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TITOLO TESI

FISH QUALITY ASSESSMENT THROUGH THE APPLICATION OF CHEMICO-PHYSICAL, SENSORY AND MICROBIOLOGICAL ANALYSES

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Abstract

The quality of fish products is indispensably linked to the freshness of the raw material modulated by appropriate manipulation and storage conditions, specially the storage temperature after catch. The purpose of the research presented in this thesis, which was largely conducted in the context of a research project funded by Italian Ministry of Agricultural, Food and Forestry Policies (MIPAAF), concerned the evaluation of the freshness of farmed and wild fish species, in relation to different storage conditions, under ice (0°C) or at refrigeration temperature (4°C).

Several specimens of different species, bogue (*Boops boops*), red mullet (*Mullus barbatus*), sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*), during storage, under the different temperature conditions adopted, have been examined. The assessed control parameters were physical (texture, through the use of a dynamometer; visual quality using a computer vision system (CVS)), chemical (through footprint metabolomics 1H-NMR) and sensory (Quality Index Method (QIM). Microbiological determinations were also carried out on the species of hake (*Merluccius merluccius*).

In general obtained results confirmed that the temperature of manipulation/conservation is a key factor in maintaining fish freshness. NMR spectroscopy showed to be able to quantify and evaluate the kinetics for unselected compounds during fish degradation, even *a posteriori*. This can be suitable for the development of new parameters related to quality and freshness. The development of physical methods, particularly the image analysis performed by computer vision system (CVS), for the evaluation of fish degradation, is very promising. Among CVS parameters, skin colour, presence and distribution of gill mucus, and eye shape modification evidenced a high sensibility for the estimation of fish quality loss, as a function of the adopted storage conditions. Particularly the eye concavity index detected on fish eye showed a high positive correlation with total QIM score.

List of papers

This thesis is based on the work contained in the following papers, referred to in the text by their Roman numerals. The papers are attached as appendix at the end of the thesis.

Paper I - Nikzhad H., Rocculi P., Romani S., Balestra F., Dalla Rosa M., Capozzi F. Red mullet (*Mullus barbatus*) freshness assessment at two different storage temperatures: comparison among sensorial and physical analyses.

Paper II - Rocculi P., Panarese V., Nizkad H., Dalla Rosa M. (2012) Isothermalcalorimetry to predict the stability of fresh fish fillets in modified atmosphere packaging (MAP). Living & Breathing Calorimetry Congress, 21th of November, GSK Ware - UK. 'Best Poster Price' for the innovative approach to isothermal calorimetry.

Paper III - Picone G., Ciampa A., Marcolini E., Vallicelli M., Dalla Rosa M., Rocculi P., Nikzad H., Bordoni A., Di Nunzio M., Laghi L., Capozzi F. (2012) A foodomics approach to evaluate the freshness of selected fish species in different aquaculture systems. In: Abstract Book of the 11th International Conference on the Application of Magnetic Resonance in Food. Vol. 1, p. 4, Wageningen: s. n, 26-29 June, Wageningen (Netherlands).

Paper IV - Ciampa A., Picone G., Laghi L., Nikzad H., Capozzi F. (2012) Changes in the amino acid composition of bogue (*Boops boops*) fish during storage at different temperatures by 1H-NMR spectroscopy, Nutrients, 4, 542-553.

Paper V - Serratore P., Nikzad H., Zavatta E., Piraccini S., Ciaravino G., Trentini M. (2011). Microbiological effects of on-board fishing vessel handling in *Merluccius merluccius*. Italian Journal of Food Safety, 1(0), 135-140.

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1. Introduction and objectives

Nowadays, fish products freshness and quality has become the key strategy for the fish industry. Consumers are increasingly aware of fish benefits for human health, and always ask for high quality products. As a support fish quality through the whole production chain is the major challenge for fish retailers particularly for fresh raw fish.

The fresh raw fish go quickly to alteration phenomena due both to the effect of endogenous enzymes and microbial activity. To ensure freshness, it is essential that the fish caught is handled in proper hygienic conditions and stored at optimum temperature (as is indicated in Regulation (EC) No. 853/2004), until the product consumption.

Freshly caught products are very sensitive to rapid deterioration, especially when stored at a not suitable temperature. Preservation under ice is one of the most efficient ways to slow the deterioration phenomena.

The rate of deterioration during storage in ice varies for each species and depends on the concentrations of substrates and metabolites and in tissue, microbial contamination and storage conditions after the catch (Pacheco-Aguilar et al., 2000). The storage period reflects the susceptibility of fish spoilage. Most of the traditional methods used to assess the quality of fresh fish, measure or evaluate the parameters that change, disappear or are formed during the fish deterioration. These techniques can be divided into sensory, physical and chemical methods; it can also be important to assess the evolution of the associated microbial component (microbiological methods).

Some of these approaches are sensitive only in later stages of advanced spoilage. Trimethylamine (TMA) is formed from enzymatic decomposition and bacterial use of trimethylamine oxide, a naturally occurring osmo-regulating substance found in most marine fish species.

Total volatiles base nitrogen (TVB-N), is an index considered unreliable for the measurement of spoilage during the first 10 days of chilled storage of cod, as well as of several other species (Huss, 1995).

TMA and TVB-N values are only useful for advanced spoilage and they begin to increase after time-temperature abused conservation (Baixas-Nogueras et al., 2003; Oehlenschlager and Sorensen, 1998).

In addition to the increase in demand, in the last years there has been an intense increase of fishery products farming involving many Asian and European countries. An

important part of the fish marketed at Europe comes from not industrialized countries, which often are lack of appropriate food control systems.

In this direction, spoilage is an important criterion for determining the overall quality of seafood products. Considerable effort has been expended in the searching of suitable methods to assess freshness while the product is still edible (Pivarnik et al., 1990). Although the sensory method is still the most satisfactory way of assessing the freshness of fish, for its limitations, its use is potentially restricted to monitor fish processing (Alasalvar et al., 2001). In addition trained panels are generally expensive, time consuming, and not always available along the different steps of the fishery chain. Consequently, to satisfy the need for quality measurements in the fish industry, instrumental rapid and/or non-destructive methods are needed (Macagnano et al., 2005), that have to be applicable at any time, along the production chain.

Several instrumental techniques have been introduced in the recent past to measure physical, chemical and biological parameters of fish such as spectrophotometers, texturemeters, image analysers, colourimeters, devices to test surface characteristics, electrical properties and electronic noses (Macagnano et al., 2005).

The aspect and colour of raw fish are the first quality parameters evaluated by consumers. In fact, in the freshness evaluation scheme submitted by Reg. (EC) NO. 2406/2004 and the Quality index method (QIM), two important points for the assessment of raw fish quality are the appearance of skin and eyes. Colour changes allow to detect certain anomalies or defects that food items may present.

The determination of colour can be carried out also by using a *tristimulus* colour measuring instrument while computer vision system (CVS) can permit the assessment of both chromatic and geometric properties.

Also the texture is a very important quality attribute of fish muscle strictly connected with fish flesh that can undergo deterioration because of inadequate storage conditions. Changes in texture of fish during storage can be measured by texture analyzers. Concentration and the pattern of free amino acids are also very sensitive to the changes occurring in fish muscle during storage. The amino acid composition of fish muscle proteins, known from long time, is remarkably constant across different species of fish (Bramstedt, 1962). As free amino acids are key molecules in both autolysis and biological spoilage reactions, their observation may offer alternatives to the K index and its variants to follow fish quality loss during storage. Proton nuclear magnetic resonance (1H-NMR)

can be in turn a suitable technique for this purpose, as suggested by recent papers published by some of the authors of the present work (Capozzi et al., 2008).

The objective of this thesis was to study the response of raw fish in terms of quality and freshness after handling/storage at different conditions through the application of a multi-analytical approach. In particular the research activity has been focused on the ability of instrumental methods, based on different physical or chemical sensors, to provide objective measures for assessing the quality of fish.

2. Composition and nutritional aspects of fish

For their nutritional characteristics, fish holds a very important role in the human diet. Many studies showed that fish consumption is associated with reduction in blood pressure (Connor, 2000) and risk of coronary heart disease (He et al., 2004). These health benefits are associated with the consumption of fish oil containing polyunsaturated long chain omega-3 and omega-6 fatty acids (Lara et al., 2007). Fish contains all the essential amino acids as lysine, methionine and cysteine being present in significant levels (Ababouch, 2005). These amino acids play a major role in maintaining health and vitality (Usyduset al., 2009). The amino acid composition of fish muscles is also associated with the functional properties of fish protein. It was reported that lysine and threonine exhibit high water solubility, making them interesting for technological reasons (Taskaya et al., 2009). The amino acid composition of fish flesh is similar to that of hen's egg, and the consumption of fish together with products of plant origin which are poor in some amino acids (lysine, threonine), enables not only a complete utilization of plant proteins, but also improves the content of the diet (Bykowski and Dutkiewicz, 1996).

The three important components of fish are proteins, lipids and water but the nutritional profile of fish is defined mainly by proteins and lipids, whereas carbohydrates are detected at very limited levels. Vitamin content is comparable to that of mammals, except for vitamins A and D, which are found in large amounts in the meat of fatty species, especially in the liver of species such as cod and halibut. As for minerals, fish meat is a particularly valuable source of calcium and phosphorus as well as iron, copper and selenium (FAO 2013).

These components are subjected to change due to the availability of food, swimming activity during intense migratory movements and metabolic changes typical of fish during their reproductive period (Ackman, 1995).

Proteins

Proteins are the major organic material in fish tissue. Fish is a rich source of proteins for human consumption, in varying proportions, depending on the species (Shahidi, 1995). All proteins, including those from fish, are chains of chemical units linked together to make one long molecule. These units, of which there are about twenty types, are called amino acids, and certain of them are essential in the human diet for the maintenance of good health (FAO 2013).

Among the proteins contained in the edible portion of fish can be distinguished sarcoplasmic myofibrillar and stromal proteins that, depending on the species, contribute in a variable percentage to the total protein content. Those sarcoplasmatic, comprising mainly albumin and proteins with enzymatic function, constitute about 26-30% of the proteins of the muscle of fish and they are particularly large on pelagic species. Those myofibrillar (actin, myosin, actomyosin, tropomyosin) constitute the majority of intracellular proteins. These proteins vary mainly during the onset of rigor mortis, the resolution of rigor and freezing long term, and they are those that give to the fish its technological properties, such as the consistency and texture of the flesh, fundamental in defining the quality of fresh fish products, but also in those processed (Shahidi, 1995). The extracellular proteins (stromal), insoluble in saline solutions, which fall within the composition of the membranes and tendons (collagen, elastin, keratin, etc..), are present in the muscles of the fish in varying percentages from 0.2 to 3%. They are responsible also of the meat consistency and in cephalopods determine the characteristic hardness after cooking process (Shahidi, 1995).

Water

Water is the principal component of fish flesh, which usually accounts for about 80% of the weight of a fresh white fish fillet. Whereas the average water content of the flesh of fatty fish is about 70% (FAO 2013).

Generally the water content of the whole body and the edible portion tends to vary in an inversely proportional manner to that of lipids (Jobling et al., 1998). Water determines fish physical characteristics, technological properties, microbial stability and of the consequent shelf-life and quality of fish and fish products. The water in fish muscle may be divided in:

- bound water, tightly bound to the proteins in the structure in such a way that it cannot readily be expelled even under high pressure, and it is also very resistant to freezing and heating. The change in the amount of bound water is very modest, even if it is the most affected by the rigor process and the conversion of muscle to meat. This water can eventually escape as drip loss (Offer and Knight, 1988).

- free water, mainly held by weak surface forces and it is not readily seen in pre-rigor meat, but can gradually develop during and after the rigor process (Fennema, 1990).

The increase in the content of free water is usually found in the edible portion, even with the passing of fresh fish storage time, for the activation of pH dependent proteases. These enzymes degrade the muscle protein components; determining a release of water (Shahidi, 1995). The increase in tissue water, corresponding with the reduction of lipid content, result in variations of consistency, elasticity and texture of the meat, that can adversely affect the appearance of the fish products and their overall quality (Torrisen et al., 2001).

Lipids

The beneficial health effects of seafood are attributed to their lipid components, particularly to long-chain polyunsaturated fatty acids of the omega 3 family (Shahidi and Cadwallader 1997). This is because humans cannot easily synthesize these fatty acids and must acquire them through the diet.

The component of lipids in fish is:

· deposit lipids, which are essentially constituted by triglycerides;

· cellular lipids such as phospholipids, glycolipids and cholesterol, which have a structural function.

The content of lipids in the tissues of fish is strictly species-specific; it depends on many factors related to the biology and the ecology of the species (Ackman, 1995). These factors may be also: the age of the subject, seasonality, the availability of food waters in which it lives and the physiological conditions at the time of capture (e.g. during the period of reproduction).

The fish lipid components are different from those present in other food, such as meat, for the greater percentage of polyunsaturated fatty acids, in particular of the series ω-3 (PUFA), to which belong the eicosapentaenoic acid (EPA, C 20:5 ω-3) and docosahexaenoic acid (DHA, C 22:6 ω-3). These, components present a beneficial effect on human health (Ruxton et al., 2004) and counteract cardiovascular disease by preventing the formation of atherosclerotic plaques in the arteries.

3. Fish freshness and quality

Among consumers of fresh fish, there is an ever increasing requirement for quality. Achieving high quality standards is the main goal of any food chain. In the case of fish products that aim is indispensably linked to the freshness and the appropriate storage conditions of raw material.

The quality attributes include a series of parameters related to safety, nutritional quality, availability, freshness and edibility (Bremner et al., 2000), which may be affected mainly by handling, processing and storage procedures from catch to consumers. Practically, physical, chemical, biochemical and microbiological changes occurring post-mortem in fishes, result in a progressive loss of food characteristics in terms of taste and a general concept of quality (Olafsdóttir et al., 1997). The binomial storage temperature and time of fish is a key factor for the final quality of the product. Fish spoilage depends mostly on temperature, which controls to a large extent the bacterial and the autolytic breakdown. Moreover, the rate of spoilage depends on several factors such as the kind of fish species and the sanitary conditions on board. Fish freshness is the most required property from the consumers because of its strong relationship to the taste (Macagnanoa, et al., 2005).

3.1 Fish safety and risks promoted by deterioration

The fish products, into account of their high nutritional value, occupy an important position in human nutrition. The consumption trend of fish products is winning by increasing amounts, not only in Europe, but also in countries traditionally less dependent on this food source. In some countries, like Japan, they are the primary protein source.

Diseases related to the consumption of seafood may be caused by a variety of pathogens: aquatic biotoxins, biogenic amines, bacteria, viruses and parasites (Gram and Huss, 2000).

In recent years the demand for fresh fish products by the consumer is increasing continuously. At the same time, due to the fishing, the availability of many species is declining. This phenomenon stimulates the trade at international level.

It is important to know that a great quantity of fish at the international trade comes from non-EU countries (as seen in **Figure 3.1**) that, in most cases, do not have well developed control systems of foods, and often have a high incidence of gastrointestinal diseases.

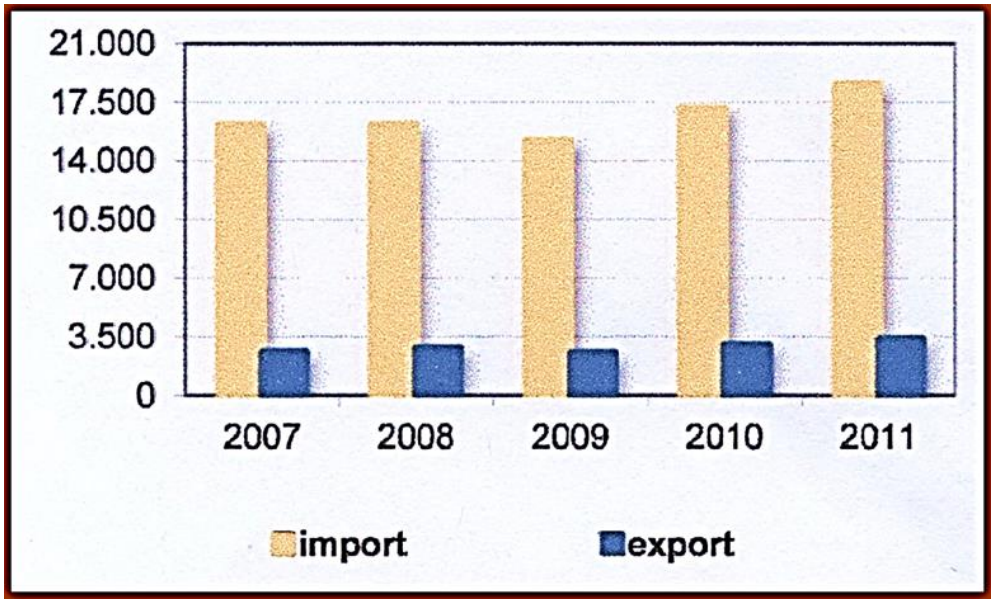


Figure 3.1. Import - Export (non-EU) of the EU-27 fish (000 t). Fish, shellfish and other aquatic invertebrates and their preparations. Source: Based on Euro stat data Ismea.

The degradation process in fish is carried out at first by muscle enzymes and later by microbial enzymes (Whittle et al., 1990; Olafsdóttir et al., 1997).

Bacterial growth during storage increases with handling and due to the direct contact with decks, equipment and boxes (Huss et al., 1974; López-Caballero et al., 2002). In a first step, bacteria present in gills, gut and skin metabolism low-molecular-weight compounds, producing volatile compounds associated to spoilage (Barros-Velázqueza et al., 2008). With the recent trend of fresh seafood consumption in the European countries, public health concerns have become an issue requiring careful attention, not only to ensure product quality but also its safety (Barros-Velázqueza et al., 2008).

To ensure the excellent quality and shelf-life, it is essential that the fish just caught are treated in an appropriate way (condition) until consumption of the product. For this reason and in order to slow down the process of deterioration, the fish must be chilled immediately after capture. Every inadequately manipulation and treatment may increase the speed of the deterioration and loss of freshness. The fish must firstly appear in impeccable sanitary conditions; in other words, the product must not in any way harm the health of the consumer.

3.1.1 The microbial flora associated with fish

Pathogenic bacteria are defined as those bacteria that may cause illness in humans. Some pathogenic bacteria are transmitted to humans via food. The bacteria in the marine environment have a complex biological role, substantiated by their different function sin the food chain. In fish, there are two groups of microorganisms:

- the indigenous microflora or native.
- the exogenous microflora or allochthonous.

In temperate or warm water fish, indigenous microbial flora consists largely of more mesophilic Gram-negative bacterial species (*Pseudomonas spp., Moraxella, Acinetobacter, Flavobacterium, Xanthomonas, Vibrio spp.*) and Gram-positive (*Bacillus, Corynebacterium, Micrococcus* and other *coccacee*, lactobacillus) (Gram and Huss, 1996).

In fish caught in cold water, however, it seems that the predominant microflora is constituted by Gram-negative species psicrotrofe in the mucus surface (mainly *Pseudomonas*, *Alteromonas* and *Shewanella*) and Gram-positive in intestinal contents (*Clostridium spp.*) (Gram and Huss, 1996).

Fish meat is believed to be the most rapidly perishable than other protein sources. The rate of decomposition is affected by different factors, such as the number and type of organisms associated with meat and by the conditions of storage, including temperature.
It is generally accepted that the flesh of the healthy live or newly-caught fish is sterile (Donn and Hackney, 1991), and the natural bacterial flora present on the surface slime of the skin, on the gills and in the digestive tract (Baross and Liston, 1970). Bacterial loads on surfaces (skin) of fish from catch can range from hundreds up to millions per square centimetre (10^2–10^7/cm^2); and in the gills and intestines in the range of 10^3 – 10^9/g (Shewan, 1962; Liston et al., 1976; Adams and Moss, 2008).

The microbial condition of fish is influenced by environment condition and the microbial quality of the water, including temperature, natural bacterial flora, salt content and so on (Feldhusen, 2000).

Pathogen microorganisms may act directly on target organs or through their toxic products or a combination of both them. Food Zoonoses occurs after ingestion of food which containing live microorganisms. The microorganisms, once in the gut, multiply causing infections (may or may not produce toxins).

About one-third of the world's food production is lost annually as a result of microbial spoilage (Lund et al., 2000). When a product rejected based on sensory assessment, the microflora can be constituted of a mixture of species, the so called spoilage microflora. The spoilage microflora can be tested for their ability to produce the compounds associated with spoilage.

Microbiological spoilage reactions in seafood depend on the initial composition or fish species, original environment and storage conditions (Huss et al., 1997, Gram et al., 2002). Spoilage bacteria dominate and contaminate the flesh/muscles through damaged parts of flesh causing the rapid spoilage of fish. Spoilage of fish is mainly due to the activity of psychrotrophic gram-negative bacteria, such as *Shewanella putrefaciens* and *Pseudomonas spp*. The process of spoilage of fish stored in ice is quite similar and is caused in large part by *Pseudomonas spp.* and *Shewanella putrefaciens* (Barile et al., 1985). *Pseudomonas spp.* are the specific spoilers of iced stored tropical freshwater fish (Lima dos Santos, 1978; Gram et al., 1990) and are also, together with *S. putrefaciens*, spoilers of marine tropical fish stored in ice (Gillespie and MacRae, 1975; Gram et al., 1990). At ambient temperature, motile *Aeromonas* are the specific spoilers at aerobically stored freshwater fish (Gorzyka and PekPoh Len, 1985; Gram et al., 1990).

The detection of specific spoilage organisms (SSO) such as *Shewanella putrefaciens*, *Photobacterium phosphoreum* and *Pseudomonas spp.* is considered more reliable than total viable counts (TVCs), for accurately assessing the freshness or spoilage level of fish and fish products (Dalgaard, 2000a; Gram et al., 2002). In **Figure 3.2** the results of correlation of TVCs and SSO in seafood, with the degree of freshness or remaining shelf life, are reported.

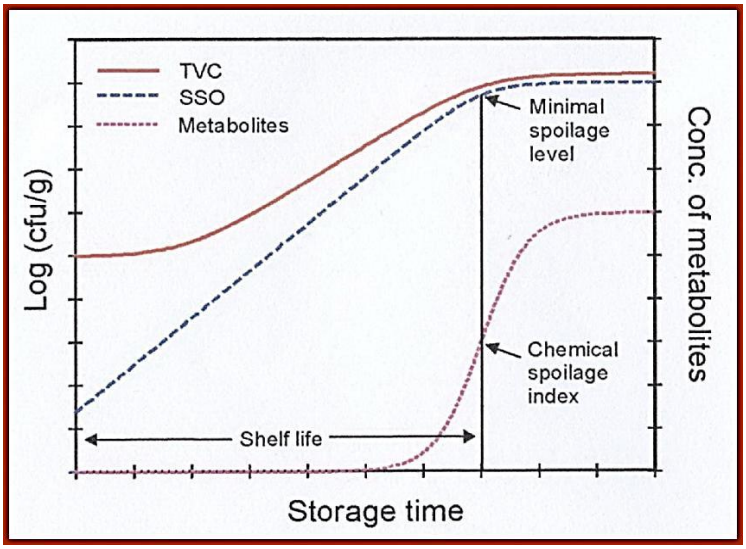


Figure 3.2. Specific spoilage organism (SSO) concept. Typical changes in total viable counts (TVCs), specific spoilage organisms (SSO) and metabolites produced by (SSO) during storage of fresh seafood (Dalgaard, 2000b).

In **Paper V**, Spoilage bacteria as Total Bacterial Count (TBC) and specific spoilage bacteria as Sulphide Producing Bacteria (SPB) were enumerated in European hake (*Merluccius merluccius*) flesh, as Colony Forming Units (CFU/g) on Plate Count Agar and Lyngby Agar at 20°C for 3-5 days.

These bacteria are able to favor the spoilage because of two important characteristics: a) they are psychrotrophic, therefore multiply at refrigeration temperatures. b) they are able to metabolize various substances present in the fish muscle, transforming them into by products associated with bad odors.

These compounds are: methylmercaptan, dimethyl sulphide, dimethyl-trisulfide, 3-methyl-l-butanal, trimethylamine, ethyl-acetate, ethyl-butyrate and ethyl-hexanoate (Miller et al., 1973a, 1973b; Ryser et al., 1984).

In particular, trimethylamine oxide produced (TMAO) content in the muscle of fish helps to produce the typical ammonia smell of the fish deteriorated.

Even in tropical and sub-tropical species, where the expected amount of *Pseudomonas spp.*is minimal, it is typical of the bacterium spoilage.

The study reported on **Paper I** enabled to draw some interesting indications about microbiological quality of hake meat (*Merluccius merluccius*).The estimated Sulphide Producing Bacteria (SPB) resulted a useful parameter to get information on the spoilage state of fish.

3.1.2. Virus

Viruses are the most numerous and diverse group of microbes in the marine environment (Shuttle, 2007). Hepatitis A, norovirus and calicivirus are relevant to human health and are isolated, where they live, in marine organisms.

Norovirus is one of the most common shellfish-borne viruses (Le Guyader et al., 2006). Norovirus appears to be the most common cause of gastroenteritis in humans word-wide and it is highly infectious (Glass et al., 2000). It is usually spread through the faecal-oral route by the ingestion of contaminated food or water (Atmar and Estes, 2001). Also epatite A and calicivirus are relevant to public health.

These viruses are isolated, in fish products, particularly in shellfish that filter, retain and concentrate the viral particles and may involve a large number of people. Outbreaks of viral enteric diseases, mainly hepatitis A and gastroenteritis, associated with shellfish consumption constitute a major health problem worldwide (Lees D, 2000).

3.1.3 Parasites

The parasite is an organism that lives on or in an organism of another species, known as the host (in this case, fish), from the body of which it obtains nutriment.

The man fell ill after consuming the flesh offish that contain live parasites that cause zoonoses. These diseases are known with the name of parasitic zoonoses of marine animals, and parasites that cause it are called "fish parasites zoonotic".

The presence of a large number of parasites in the flesh of the fish causes severe and obvious alterations of the organoleptic characteristics (texture, colour, odor, etc.).

In these cases, the fish product often becomes not suitable for human consumption and it takes a look repulsive.

The most common parasite that causes zoonoses are two Anisakid nematodes:

- *Anisakis simplex* (**Figure 3.3**).
- *Pseudoterranuova decipiens* (**Figure 3.4**).

Anisakis is a genus of parasitic nematodes, which have life cycles involving fish and marine mammals (Berger et al., 2006). They are infective to humans and cause anisakiasis.

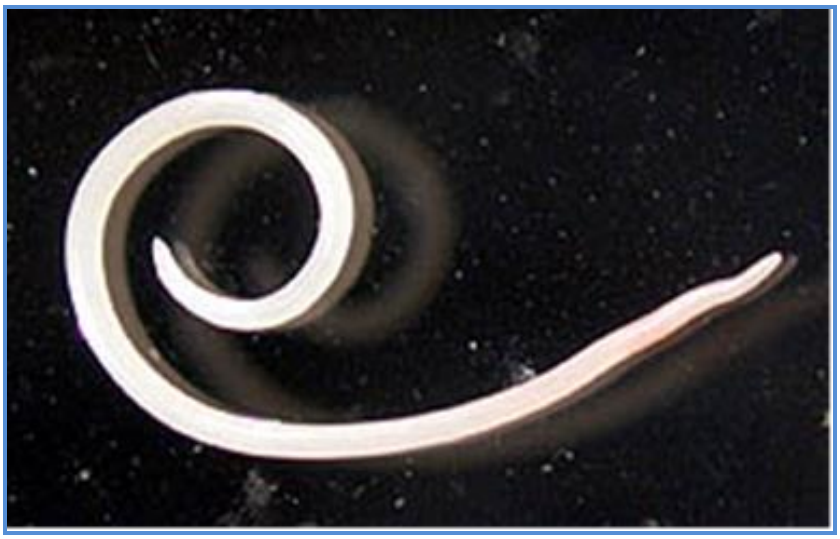


Figure 3.3. *Anisakis simplex*

Figure 3.4. *Pseudoterranova decipiens*

Anisakiasis poses a risk to human health through intestinal infection with worms from the eating of under processed fish, and through allergic reactions to chemicals left by the worms in fish flesh (Amato Neto et al., 2007). It is a human parasitic infection of the gastrointestinal tract caused by the consumption of raw or undercooked seafood containing larvae of the nematode *Anisakis simplex*. The first case of human infection by a member of the family Anisakidae was reported in the Netherlands by Van Thiel, who described the presence of a marine nematode in a patient suffering from acute abdominal pain (Audicana et al., 2008). It is frequently reported in areas of the world where fish is consumed raw, lightly pickled or salted (Audicana et al., 2008).The areas of highest

prevalence are Scandinavia (from cod livers), Japan (after eating sushi and sashimi) and the Netherlands (by eating infected fermented herrings).

Within hours after ingestion of infective larvae, violent abdominal pain, nausea, and vomiting may occur. Occasionally, the larvae are regurgitated. If the larvae pass into the bowel, a severe eosinophilia granulomatous response may also occur one to two weeks following infection, causing symptoms mimicking Crohn's disease.

Diagnosis can be made by gastroscopy examination, during which the 2-cm larvae are visually observed and removed, or by histopathology examination of tissue removed at biopsy or during surgery.

Salting and marinating will not necessarily kill the parasites. Many countries require all types of fish with potential risk intended for raw consumption to be previously frozen to kill parasites. The disease can be prevented through:

- health education (raising consumer and producer awareness about the existence of anisakis worms in fish is a critical and effective prevention strategy);
- evisceration after fishing to prevent the migration of larvae in muscle;
- visual control;

The freezing of fishery products, conducted at a temperature of –20°C for at least 24 hours (Regulation EC No. 853/2004), is recognized as an effective way to kill the majority of fish parasites. Also the use of high temperatures, such as the cooking of fish, isa solution for the elimination of these parasites.

3.2 Quality degradation of fish

Degradation in fish products are caused by changes that are induced by oxidation reactions, from reactions due to specific enzymes of the fish or to the metabolic activities of the microorganisms.

The post-mortem changes that lead to the deterioration depends not only on the chemical composition of the fish and its microbial flora, but also extrinsic factors (Ashie et al., 1996). At the time of fishing is generally admitted that the meat is sterile and the microbial flora is localized exclusively on the skin, on the gills and in the digestive tract (Donn and Hackney, 1991). These bacteria significantly outweigh the Gram-negative psychrophilic, including Pseudomonas, Vibrio, Moraxella/Acinetobacter, Flavobacterium/Cytophaga, Aeromonas, while Gram-positive, bacteria such as Clostridium are less represented (Liston, 1980). The passage of bacteria to the muscular masses occurs following the rupture (breakdown) of the membrane coelomic, and migration from

the outside (skin and gills). Such contamination is favored by the conditions of handling and the use of improper temperatures, favoring a more rapid deterioration.

Most of the changes that occur during storage of fish are derived by bacterial (Shewan, 1977; Linston, 1980; Hobbs and Hodgkiss, 1982). The occurrence of undesirable odours and flavours are due mostly to the bacterial metabolism of proteins, amino acids, nitrogenous bases etc.

In the immediate post-mortem, in products of animal origin, the phenomena of alteration begins; it occurs at a speed in relation to the activity of endogenous enzymes and microbial contaminants.

Among animals, the fish is undoubtedly the least preservable due to the speed and intensity with which undergoes deterioration. This is favored by the particular chemical composition and physiological characteristics of marine animals. Synthetically can be marked as following factors:

• The endogenous enzymes are able to act at temperatures lower than those of terrestrial animals, also have a high lytic-capacity having to ensure, in particular in fish predators, digestion of prey not chewed.

• The meat has a high content of non-protein nitrogenous substances that provide an excellent substrate for the bacterial growth.

• The low concentration of carbohydrates disadvantage the acidification post - mortem and consequently the pH does not drop below 6.2 to 6.5, values close to neutrality that are favorable to the development of bacteria.

• In the blue fish, a name that combines species that are very different from each other, high amounts of polyunsaturated fatty acids are present, chemically more reactive than saturated fatty acids, and therefore more exposed to oxygen which causes the fast incidence of rancidity.

• The predominance of psychrophilic bacteria such as Pseudomonas spp. and Vibrio spp., those are able to grow well even at low temperatures.

• The trimethylamine oxide (TMAO), present in a large number of marine fish (Cappelli and Vannucchi, 2005). Development of TMA depends on the content of the substrate trimethylamine oxide (TMAO) in the fish. TMAO can be split into dimethylamine and formaldehyde by enzymes tissue. The bacteria, such as Aeromonas putrefaciens are able to use the TMAO, as the last hydrogen acceptor, and therefore capable of growing

even in microaerophilic environment, i.e. in the absence of oxygen producing trimethylamine (TMA).

Extrinsic factors that may be involved in phenomena after death of fish include:

- season and temperature of the water harvesting fish;
- fishing methods;
- stress, which results in less post-mortem acidification of the meat;
- injury on the surface;
- habitat, water collection;
- salt content of the water;

3.2.1 Problems of contamination in fish products

Anyone who works in the food industry and catering should never underestimate the importance of hygienic-sanitary aspects. They have to be observed precise norms and behaviors, to allow for preventing all forms of risk.

Food contamination, due to handling and poor storage, can occur at any stage between production and consumption, so the respect of a perfect food hygiene must begin with the processing of raw materials and continued until the distribution and final consumption of food.

The various systems for storage or packaging of seafood have one purpose: to ensure that the product retains, as much as possible unchanged, its organoleptic and nutritional characteristics over time. However we must not forget that any food must first appear in sanitary conditions impeccable (unexceptionable). In other words, the product must not in any way harm the health of the consumer (Uniprom 2013). In addition to microorganisms which derive from the environment (water), there are many pathogenic microorganisms that may be associated with the consumption of seafood. These microorganisms may also be conveyed by a post-mortem contamination due to processing and treatments improper, from the sanitation point of view.

In the case of fish products, to ensure the excellent quality and shelf-life, it is essential that the freshly caught fish are treated in an appropriate manner. Some of these treatments include post-harvest peeling, washing, gutting, decapitation and bleeding. Bad handling practices may, however, increase the threshold of deterioration. The correct gutting and decapitation may contribute to the extension of the shelf-life. The main advantage of gutting is to prevent spoilage due to autolysis, rather than due to microbial

action. The FAO (1973) states that inadequate gutting may be worse than not gutting at all, because facilitates the spread of microorganisms in the flesh of fish.

3.2.2 Sanitation problems related to the method of fishing

The initial quality of fresh fish is affected by the method of capture (Shewan, 1971). A wide variety of fishing techniques is used to catch the fish for consumption; these include traps, hooks, pots, and a wide variety of nets (Alverson, 1976).

From the point of view of contamination, Shewan (1949) reported that in general trawled fish carry microbial loads 10 to 100 times higher than line-caught fish, because of mud stirring contamination and gut contamination produced by the pressure of the fish in the net.

In the trawl survey carried out with pelagic (midwater trawling), the catch is damaged according to the species and quantity, with phenomena of asphyxia and crushing (bleeding or laceration of the skin and the abdominal wall, possible leakage of stool). Cod and blue fish have a skin shortly consisting, for which they are exposed more easily than other fish species in this type of alterations. In bottom trawling, the material on the bottom and the mud leads to harm ever greater with an increase of contamination than other types of fishing.

In the small fishing boats, generally do not have a cold room (the length of the fishing for these boats is a few hours). The fisherman, before leaving port for the fishing trip, must be supplied with ice to chill the catch. This practice is not unusual at the summer (Uniprom 2013).

In fishing with lines, the fish quality changes are also related to the stress phenomena. In sport fishing for tuna, the stress caused by a prolonged recovery during fishing and the partial denaturation of myofibrillar proteins can promote softening of fish muscles, having concomitant colour loss and less ability from the muscle to retain water.

In gillnetting, for a prolonged time, is susceptible to changes in the freshness of fish, especially in hot weather, causes discoloration of the skin and gills, and limitation of acidification of the meat with consequent limitation of rigor and decrease of the subsequent shelf-life of the product (Uniprom 2013).

3.2.3 The problem of histamine and other biogenic amines

The experience gained over time lead us to conclude that a small part of histamine develops any way in the tissues of fish, precisely by means of endogenous enzymes; this is a relatively small portion, about 10% of the whole quantity. However most of the histamine is produced by bacteria that proliferate in the food (Uniprom 2013).

As we have remarked before, the origin of biogenic ammines is only partly due to autolytic phenomena in the tissue of origin. In fact it is of bacterial origin, being due to the action of specific enzymes, produced by numerous microorganisms, on free amino acids generated during the first step of degradation processes. The activity of these enzymes (primarily the histidine - decarboxylase) is a function of several factors, first of all the free content of histidine in the food substance. The highest levels of histidine were found in muscle tissue of some fish species of red meat, belonging to the families *Scomberesocidae, Scombridae* (tuna, yellowfin tuna,bonito, mackerel), and *Clupeidae Engraulidae* (sardines, anchovies, herring), at intracellular level as well as in the blood.

The highest levels were observed, in descending order, on tuna, mackerel, sardines and herring. Under favorable conditions, in these species, microbial activity may rapidly induce the synthesis of large amounts of histamine (1%) that do not involve appreciable organoleptic changes, but they can cause serious consequences for the consumer.

Even in fish with white meat can be observed the formation of histamine, but in this case the causes seem to be ascribed mainly to environmental factors such as an imperfect prolonged conservation (Uniprom 2013).

Although constituting the primary requirement for the action of bacterial decarboxylase, the presence of the substrate is not the only factor for the formation of histamine. Of fundamental importance is the processing/distribution temperature, which greatly influences the amount of amine produced. The delay in cooling and high temperatures is of particular importance for the species of the mackerel family (tuna, mackerel). However the low temperatures are able to slow down substantially the bacterial synthesis of histamine (Uniprom 2013).

Many researchers agree that the correlation between the production of amines and the state of preservation not only involves istamine, but also cadaverine, putrescine, spermidine and spermine. Moreover, histamine still seems to be the only molecule responsible for the phenomena of intoxication (sgombroid poisoning), although other biogenic amines play a synergistic or potentiating role.

The bacteria that are responsible for the production of high levels of histamine are those which possess the enzyme commonly istidine-decarboxylase, and are essentially the *enterobatericee* and *pseudomonadacee* (among strongest producers of histamine *Morganellamorganii* and *Klebsiellapneumoniae*, *Proteus*, *Pseudomonasspp*can be mentioned), but also among the Gram-positive bacteria species capable of producing histamine have been identified (some various species of *Bacillus* and *Micrococcus* and *Lactobacilli*). Previous studies showed that the causes prevailing in cases of intoxication are contamination with *Morganellamorganii*, together with the storage temperature of the fish and to the interval time between the capture and the refrigeration of fish (Uniprom 2013).

3.2.4 Marine Toxins

Illness from seafood poisoning were caused by dangerous contaminated chemicals or marine biotoxins (FAO 2004; Campas 2007). Seafood contaminated by marine biotoxins apparently look, smells or tastes normal but after human or animals eat the seafood, they may suffer a variety of gastrointestinal and neurological illnesses (Osek 2006).

The main toxins associated with the consumption of fish products are:

- Paralitic Shellfish Poisoning (PSP);
- Diarrhetic Shellfish Poisoning (DSP);
- Neurotoxic Shellfish Poisoning (NSP);
- Amnesic Shellfish Poisoning (ASP);
- Ciguatera Poisoning (CFP);

Marine toxic compounds are produced by phytoplankton or their associated bacteria. Well documented is the process through which shellfish can accumulate marine biotoxins in their edible tissues from harmful algae. Marine phytoplankton toxins can cause finfish mortality and shellfish toxicity, as well as poisoning or death of humans, marine mammals and seabirds that ingest contaminated fish or shellfish (Anderson and White, 1992; Baden and Trainer, 1993).

These toxins passed along to consumers through the consumption of shellfish and arrived in gastrointestinal tract. In recent years marine biotoxins have been the most important responsible in poisoning associated with the consumption of fish products.

The presence of CFP is caused by the consumption of subtropical and tropical marine carnivorous fish that have accumulated ciguatera toxins through the marine food chain. The marine biotoxins are heterogeneous group with different structure. It is important to know that all algal biotoxins are resistant to heat and cannot be destroyed by cooking.

Consumption of a variety of shellfish and fish causes an increasing number of human intoxications around the world. Diagnosis depends mainly on the recognition of specific signs and symptoms and on the identification of marine toxins present in remains of the seafood involved. Monitoring seafood for toxicity is essential to manage the risks. However, there are several limitations in toxicity monitoring, such as the variation in toxin content between individual shellfish, the different detection and even extraction methods for the various toxins, and the frequency of sampling to ensure that toxicity does not rise to dangerous levels, in temporal or spatial gap between sampling times or location (FAO 2013).

4. Food safety, EU regulations and sensory quality assessment

In recent years, the hygienic-sanitary aspects related to the quality of fish products have been a topic of particular interest to the European Union. The procedures for EU food safety concern the whole production chain of the food for animal and human consumption. In this direction the European Union provides a comprehensive legislation, outlining the responsibilities of producers and suppliers to ensure the quality and safety of the food chain. The control system should not necessarily be considered a punitive value, but as a valuable tool for monitoring the market to ensure consumers.

In 2000, the White Paper on Food Safety by the European Commission was published; with its 117 points it represents a summation on food security and introduces important aspects such as:

- establishment of an 'European Food Authority (a kind of analogue of the Food and Drug Administration).

- concept of tracing the paths of food and feed ingredients throughout the entire food chain.

- concept of health and well-being of bred animals.

- concept of primary responsibility of all participants in the food chain.

The process started by the European Union, has led to Regulation (EC) No. 178/2002 and the subsequent processing of this legislation includes a series of regulations (Reg. EC No. 852/2004, 853/2004, 854/2004 and 882/2004) known as the "Hygiene Package", which came into force after 1 January 2006. Reg. (EC) NO. 178/2002 lays down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in the food industry. This regulation shall apply to all the stages of production, processing and distribution of food, places the responsibility for food safety to producers (farmers and fishermen) who have to prepare a traceability system linking in coming raw materials and products in output.

Food legislation is intended to protect the interests of consumers through the controls on the unsafe food market (harmful to health or unfit for human consumption).For this reason it is also planned the construction of a traceability system, which allows reconstruction and follow a food or feed through all stages of the production chain.

The "hygiene package" is a body of EU law laying down hygiene rules for foodstuffs produced, in the EU and non-EU countries exporting to the EU, and includes the following acts:

- The Reg. (EC) NO. 852/2004 laying down general rules of hygiene for foodstuffs, shall apply to all stages of production, processing and distribution of food and to exports, assigning primary responsibility for the safety of food business operator who is required to comply with the necessary requirements to avoid contamination of the product and to establish and implement procedures based on HACCP (identifying critical control points where there is a potential risk and establishing critical limits for these points which separate acceptability from unacceptability, applying monitoring procedures at critical points and corrective actions in cases of exceeding the critical limits). The operator must also ensure that the personnel involved in handling have undergone training on health risks; the competent authority shall verify that all stages of production, processing and distribution meet the requirements of hygiene.

Regulation (EC) No. 853/2004 laying down specific hygiene rules for food of animal origin, in order to guarantee a high level of food safety and public health.

Regulation (EC) No. 854/2004 putting in place a Community framework of official controls on products of animal origin intended for human consumption.

Official controls on products of animal origin according Regulation (EC) No. 853/2004:

- Food hygiene audits:

Food-chain information – product origin or treatment (documents);

Design and maintenance of premises and equipment;
Pre-operational and post-operational hygiene;
Personnel hygiene;
Training (hygiene, HACCP procedures);
Pest control;
Water quality;
Temperature control;

- HACCP audits:

Continuous and proper application of procedures;
Conformity of microbiological criteria;
Conformity regarding residues, contamination, prohibited substances;
Absence of physical hazards;

Regulation (EC) No. 882/2004 of the European Parliament on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.

The "hygiene package" is supplemented by other EU legislation on food safety. Still be issued and updated with other measures to control the food chain. One of the last is:

Reg. (EC) No. 1224/2009 that establishes a Community system for control, inspection and enforcement to ensure compliance with the rules of the Common Fisheries Policy.

4.1 Methods for fish freshness assessment

Most of the methods used to assess the quality of fresh fish, measure or evaluate the parameters that change, disappear or are formed during the deterioration of the fish. These methods can be divided into sensory, physical and chemical methods, and can also be important to assess the evolution of the microbial component associated (microbiological methods). In any case, the official control is still mainly based on sensory evaluation, method that lacks objective and universally valid, because the experience and entrusted to the discretion of the operator (Cianti et al., 2007).

4.1.1 EU quality grading scheme

The EU scheme is commonly used in Europe for sensory evaluation of fish (Regulation No. 2406 introduced in 1996, European Community). This scheme includes specific evaluation grades for different group of fish, respectively E, A, B and not admitted to human consumption. E (Extra) refers to the level of quality as high as possible, A (acceptable) is assigned to a product of good quality, while B (poor) is the limit (below that the product is no longer considered suitable for human consumption).

This method is widely used for a variety of fish and has been satisfactorily correlated with chemical parameters, such as volatile amines (Pérez-Villarreal and Howgate 1987), microbial counts (Pastoriza et al., 1998), and time of ice storage (Koutsoumanis and Nychas, 1999). However, its suitability has been questioned because, in using general parameters, it does not take into account particular differences between species, because only general parameters for groups of fish are used, and each table is valid for several species.

4.1.2 Quality index method (QIM)

It is very important to find good ways to monitor the freshness of the fish in order to have a measuring instrument to keep the fish at the best quality until the consumption.

Evaluation procedures for raw fish should be rapid, reliable, simple to apply, and specific for particular fish species. Quality index method (QIM) is a freshness grading system for seafood that encompasses these characteristics; it is recognized as reference method in sensory research (Olafsdóttir et al., 1997; Martinsdóttir et al., 2001). In addition, the QIM is usable in the first part of the storage period, where other instrumental methods are inaccurate (Nielsen et al., 1992).

The Quality Index Method (QIM) is a promising method to measure the freshness of fish, which shows to be both rapid and reliable (Martinsdóttir et al., 2001).

This method was originally developed by the Tasmanian Food Research Unit in Australia (Bremner et al., 1985), and it has been developed further by European fisheries research institutions.

QIM was primarily used for the evaluation of whole and gutted fish. Also for cooked fish the sensory analysis is necessary to determine the maximum storage time (Bogdanovic´et al., 2012). Up to now, QIM has been developed for different species. A part of these are for: cod (*Gadus morhua*), frozen and filleted (Warm et al., 1998), mediterranean hake (*Merluccius merluccius*) (Baixas-Nogueras et al., 2003; Triqui, 2006),

frozen hake (*Merluccius capensis* and *Merluccius paradoxus*) (Herrero et al. 2003), farmed atlantic salmon (*Salmo salar*) (Sveinsdóttir et al., 2003), whole scad (*Trachurus trachurus*) (Inácio et al., 2003), tub gurnard (*Chelidonichthys lucernus*) (Bekaert 2006), processed herring (*Clupea harengus*), stored both in air and under modified atmosphere packaging (MAP) (Lyhs and Schelvis-Smit, 2005), and raw gilthead seabream (Huidobro et al., 2000, 2001; Alasalvar et al., 2001), raw and whole herring (*Clupea harengus*) (Martinsdóttir et al., 2001; Nielsen et al., 2004). The scheme of Nielson et al. (2004) was applied by Özyurt and co-workers (2009) for red mullet (*Mullus barbatus*) and goldband goatfish (*Upeneus moluccensis*) evaluation.

The technique is based on selecting a number of significant sensory parameters (skin, eyes, gills, etc.) characteristic for a particular species and allocating scores to each attribute, depending on the state of freshness or quality of the fishery products (Martinsdóttir et al., 2001; Sveinsdóttir et al. 2003). QIM gives scores closed to zero for very fresh fish, whereas increases the scores as the fish deteriorates (Martinsdóttir et al., 2001; Huss et al., 1995). Deterioration progress with storage time would be described with maximum of 3 demerit points at spoilage, with the quality index (QI) increasing linearly with storage time in ice.

The scores for all the characteristics are then added to give an overall sensory score, called Quality Index. As no excessive emphasis is laid on a single attribute, a sample cannot be rejected on the basis of a single criterion and minor differences in results for any of the criteria do not unduly influence the total QIM score (Luten and Martinsdóttir, 1997). The aim is to achieve a linear correlation between the sensory quality expressed as the sum of demerit scores (QI) and storage life in ice, which makes it possible to predict the remaining storage life (Nielsen, 1997; Hydilg and Nielsen, 1998; Martinsdóttir et al., 2001).

5. Chemical indexes for fish freshness assessment

Most of the chemical procedures for freshness evaluation is based on the identification of one of the numerous and complex changes of fish, once starts the degradation process (Damoglou, 1979). The content in the trimethylamine (TMA) (Tozawa et al., 1971), the total volatile basic nitrogen (TVB-N) (Antonacopoulos and Vyncke, 1989), the individual nucleotides (Hattula et al., 1993; Jacober and Rand, 1982) and the nucleotide degradation product ratios (such as hypoxanthine, ratios between ATP degradation products (K values), Ki values) have been used as freshness indicators (Burns et al., 1985; Ehira and Uchiyama, 1987; Karube et al., 1984; Luong and Male, 1992; Dalgaard, 2000b).

5.1 Total volatile base nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N) is one of the most widely used measurements of seafood quality. It is a general term which includes the measurement of trimethylamine (TMA), dimethylamine (DMA), ammonia and other volatile basic nitrogenous compounds associated with seafood spoilage. Although TVB-N analyses are relatively simple to perform, they generally reflect only later stages of advanced spoilage and are generally considered unreliable for the measurement of spoilage during the first 10 days of chilled storage of cod, as well as several other species (Huss, 1995). The concentration of TVB-N in freshly caught fish is typically between 5 and 20 mgN/100 g muscle, whereas levels of 30– 35 mgN/100 g muscle are generally regarded as the limit of acceptability for ice-stored cold water fish (Huss 1988; Connell, 1995).

5.2 Trimethylamine (TMA)

Classical chemical methods for the analysis of total volatile bases (TVB) and trimethylamine (TMA) are used for the determination of fish freshness (Olafsdóttir et al., 1997). TMA production is the most commonly indexes used to evaluate the freshness of fish; this compound is produced during chilled fish storage. This compound is found in very low levels in fresh fish, and its formation is associated with bacterial spoilage (Fernandez-Salguero and Mackie, 1987). TMA is formed from enzymatic decomposition and bacterial use of TMAO, naturally occurring osmo-regulating substance found in most marine fish species (Koutsoumanis and Nychas, 1999). The quantity and presence of this compound depends on the species, size, sex, station of year, etc. (Tsigarida et al., 2003). TMA and

TVB-N values are only useful for advanced spoilage and they begin to increase after time-temperature abused conservation (Baixas-Nogueras et al., 2003; Oehlenschlager and Sorensen, 1998). However some studies have shown excellent correlation between TMA level and eating quality (including iced cod) (Wong and Gill 1987).

5.3 K-value

In fish, shellfish, crustaceans and cephalopods, ATP is rapidly degraded, after death, to inosine monophosphate (IMP) by endogenous enzymes (autolysis). The further degradation of IMP to inosine and hypoxanthine is much slower and it is catalysed mainly by endogenous IMP phosphohydrolase and inosine ribohydrolase with a contribution from bacterial enzymes as storage time increases. The degradation of ATP was found to parallel the perceived loss of freshness of fish as determined by trained panel (Gill, 1995). Therefore, the K-value gives a relative freshness rating based primarily on the autolytic changes which take place during post mortem storage of the muscle.

A shortcoming of the K-value as a freshness index is that it varies between species owing to differences in rates of ATP degradation. It also varies with post-mortem time and temperature, storage conditions, handling conditions and method of kill (Olafsdóttir et al., 1997). The K-value has been reported as not being a suitable predictor of shelf-life for CO_2 packed refrigerated striped bass strips (Handumrongkul and Silva, 1994).

Several studies suggested that none of these chemical indicators that include total base nitrogen (TVB-N), biogenic amines, trimethylamine (TMA), dimethylamine (DMA), K value, etc., are universally applicable (Gill, 1990; Botta, 1995).

6. Instrumental techniques for fish freshness assessment

In consideration that spoilage is an important criterion for determining the overall quality of seafood products, considerable effort has been expended in searching for suitable methods, with which to assess freshness while the product is still edible (Pivarnik et al., 1990).

Although the sensory method is still the most satisfactory way of assessing the freshness of fish, it has limitations and therefore its use is potentially limited in fish processing and technology sites (Alasalvar et al., 2001). In addition trained panels are generally expensive, time consuming, and not always available along the different steps of the fishery chain. Consequently, to satisfy the need for quality measurements in the fish industry, instrumental methods are needed (Macagnano et al., 2005).

Several instrumental techniques have been introduced in the recent past to measure physical, chemical and biological parameters of fishes such as spectrophotometers, texturemeters, image analysers, colourimeters, devices to test surface, electrical properties and electronic noses (Macagnano et al., 2005).

6.1 Colour

Humans perceive the world through our five senses: sight, hearing, touch, taste and smell, whose vision is usually the first signal for the detection of events and objects. The process of seeing includes many activities: the detection by the eyes, the interpretation by our brain, the recognition of movement and location of objects to their surroundings, the intensity and quality of the light and the colour appearance of objects or events in the visual scene (MacDougall, 2002). The quality of fish products is defined by the visual appearance of the products and visual appearance includes measurable parameters such colour. Colour can inform us if the fish is altered. The experiments in mixing colours clearly demonstrated that people with normal colour vision must have at least three retinal pigments in their eyes, detecting the short, mid and long wave lengths of the visible spectrum. The first truly functional system for measuring colour, as specified by the Commission International de l' Eclairage (CIE), was the so called CIE 1931 2° visual filed system of colour measurement.

With the development of computers, colour measurements and calculations are now routinely used in research and in the food industry for studies of food functionality, standardization of product ingredients and process control. The CIE system of colour

measurement transforms the reflection or transmission spectrum of objects into a three-dimensional colour space, using the spectral power distribution of the illuminant and the colour matching functions of standard observers. The original CIE 1931 Y, x, y system of colour measurement is not visually uniform. Near uniform colour spaces of practical importance, there are spaces Hunter and CIELAB. CIE L * a * b *, also known as CIELAB, has generally replaced the Hunter space for industrial application. The coordinates L * a * b * are used to define the positions of all the colours in the uniform colour space (Schubring, 2010).

The terms of colours can be divided into subjective and objective. The subjective terms (i.e. psychosensorial) are: brightness, lightness, hue, saturation, chroma and colour scheme. Colourfulness is that aspect of visual sensation according to exhibit more or leschromatic colour. Although hue is easily understood as that attribute described by colour names red, green, purple, etc.; the difference between saturation and chroma is less easily understandable. Chromatic saturation is judged in proportion to its brightness, and chroma is less easily comprehended. A similar difference exists between lightness and brightness. Lightness is the relative brightness. Lightness is not influenced by the level of illumination, because it is the percentage of reflected light, while the sensation of brightness increases with the level of illumination.

The objective terms are tied to the stimulus and are evaluated from spectral power distribution, the reflectance or transmittance of the object and the observer response. They provide the basis for the psychometric qualities that correspond most closely to those received (MacDougall 2002).

For CIELAB space, the terms are lightness (L *), hue ($h°=\tan^{-1}(a*/*b)$) and chroma ($C*= (a *^2 + b*^2)^{1/2}$. CIELAB total colour difference can be expressed as the coordinates of colour space or as lightness, hue and chroma.

Colour is the perception that comes from the detection of light after it has interacted with an object. The perceived colour of an object is influenced by three entities: the physical and chemical composition of the object, the spectral composition of the light source illuminating the object, and the spectral sensitivity of the viewer's eye. Because everyone is sensitive to food colour, the appetite is stimulated or damped in almost direct relation to the observer's reaction to colour. The colour indicates the flavor we will taste (Downham and Collins 2000). For food, consumers often evaluate the initial quality of the product by its colour and appearance. In food processing and cooking, the colour serves as a cue for doneness of foods and is correlated with changes of aroma and flavor. In

general, colour and appearance affect the sensory perceptions of the consumer (Lawless and Heymann 1998). The colour of fish and meat products is an essential factor by which consumers judge their acceptance (Clydesdale 1991).However, the colour of food is not stable, because it changes with decreasing freshness.

6.2 Computer vision system (CVS) for visual quality assessment

Photoelectric colour measuring instruments can be divided into two classes: trichromatic colourimeters and spectrophtometers. Colourimeters are *tristimulus* (three-filtered) devices that make use of filters red, green and blue that emulate the response human eye to light and colour. The modern *tristimulus* instruments are connected to computers with automatic compensation and the provision of different colour spaces. A colourimeter uses a light source for illuminating the sample to be measured. The light reflected off the object then passes through the glass filters to simulate the standard observer's functions for a particular illuminant. A photo-detector beyond each filter detects the amount of light passing through the filters. These signals are, then, shown as X, Y and Z values (Schubring, 2010).

The accuracy of predicting colour parameters from one instrument to another varies depending on the raw food. The type of sample seems to have a greater effect on the accuracy of the measurement as well as the size of the instrument's measuring area. The result change from one instrument to another is systematic and can be described by a linear regression. The regression can be used to compare colour values expected from one instrument with those obtained from another. The accuracy of prediction increases with a greater homogeneity of the food samples (Baardseth et al. 1988).

The assessment of food image characteristics (i.e. colour, size, shape and structure) have been applied widely in the food industry for quality evaluation and control of a wide variety of foods. Colour characteristics are effective tools for indicating reconstruction of the components of food products during processing.

Spaces for the hardware-orientated spaces are preferable able for observing small variations in the colour of food products during processing. With the remarkable development in hardware, the use of image data as input features could become realistic, which might be an exceptional indicator of food quality (Zheng et al., 2006).

An automated image analysis method is able to detect different visual quality parameters (e.g. cutlet area, dorsal fat depot, red muscle, fat percentage and colour of rainbow trout cutlets). It is also possible to produce images of cutlets of adequate quality

for image analysis, using a simple flatbed scanner (Stien et al. 2006a). With this simple acquisition system no elaborate lighting developed is necessary (Stien et al. 2006b). An image acquisition system allows to obtain digital images in L*a*b*units of colour for each pixel of the digital red, green, blue (RGB) image. The five numerical models developed were able to measure the colour in L*a*b* units and simultaneously measure the colour of each pixel on the target surface. This is not the case of conventional colorimeters. Among applied models the best results were obtained with the quadratic and neural network model (Leòn et al. 2006).

A simpler method used a combination of digital camera, computer and graphics software to analyze the colour of food product surface (Yam and Papadakis, 2004). Colour measurement, particularly in the L*a*b* space, provides a better correlation among groups of studied fish than sensory analysis. In fact, although in agreement with the results of the panel, the colorimetric method could distinguish all the groups in terms of mean colour and its heterogeneity. However, it has become apparent that the determination of only one quality attribute was not sufficient. Combining the data from the various sensors improves the estimate of the freshness of the fish (Ólafsdóttir et al. 2004). To demonstrate this, colour, texture and electronic nose measurements were selected, and their calibrated outputs combined to construct a so-called artificial quality index (AQI) (Di Natale, 2003). In this research it was reported that the computer visual quality analysis was able to differentiate and quantify colour distributions in fish samples with uneven colour.

In the research reported in **Paper I** image analysis performed by CVS, for the evaluation of red mullet freshness, resulted very promising. Among CVS parameters, skin colour, presence and distribution of gill mucus, and eye shape modification evidenced a high sensibility for the estimation of fresh red mullet quality loss, as a function of the two different storage conditions adopted (0 and 4 °C). Particularly the index of concavity detected on fish eye showed high correlation with sensorial eye and total QIM score.

6.3 Texture

“Texture” is an important property of fish muscle as it is a fundamental part of its quality. Fish flesh may become tough because of frozen storage or soft and mushy due to autolytic degradation (Huss, 1995).

Textural properties have been defined as a group of physical characteristics that arises from the structural elements of the food, and sensed primarily by the feeling of touch. Texture was defined as the sensory and functional manifestation of the structural

and mechanical properties of foods, detected through the senses of sight, hearing, touch, and kinesthesia (Szczesniak 1963, 2002). The organoleptic quality of fish depends largely on their structural characteristics. In this regard, the muscle of the fish is composed of fibers parallel to the longitudinal axis of the body, crossed by sheets of connective tissue, for the segments of fibre known as myotomes. The structure of the fibers is similar to that of striated muscle, typical voluntary muscle (Howgate, 1977). The main components of the proteins in the muscle are: the myofibrils proteins that constitute the myofibrils, basic contractile element of the musculature; sarcoplasmic proteins, mostly composed of enzymes and proteins of the connective tissue, consisting primarily of collagen. Both connective tissue and muscle fibers contribute to the texture of raw fish (Dunajski, 1980). An inverse correlation was found between collagen content and muscle tenderness of raw fish muscle (Hatae et al., 1986; Sato et al., 1986).

For a given species, significant positive correlations were found between the density of muscle fibers and some measured characteristics of the texture, such as chewiness and firmness (Johnston et al., 2000). Furthermore, the differences in content of the sarcoplasmic proteins that coagulate, impending the sliding of the fibers (Hatae et al., 1990), have been proposed to account the firmness of the muscle of fish.

Edible parts of fish, different from the muscles, determine structural and compositional characteristics able to make particular textural attributes to the products. There are many factors that can affect the texture of the fish, ranging from the species differences, the biological condition of the fish, or the methods authorized for capture or killing it. Particularly, after the death of the fish, the biochemical changes associated with the onset and resolution of rigor mortis appear. Before the onset of rigor muscle, fish is soft and elastic. In rigor, muscle becomes hard, due to the contraction of the fibers which constitute the complex actomyosin, and with its resolution the muscle becomes soft and less elastic. An opposing process to the stiffness occurring in rigor, called tenderization, begins within some hours post-mortem and continues during storage (Careche and Barroso, 2009).

This tenderization affects the structural key proteins in the myofibrils and the extracellular matrix, as well as proteins involved in connections myofibril-myofibril and myofibril-sarcolemma (Delbarre et al., 2006). The extent of these changes and their effect on muscle texture depend on many factors, including species, the pre-slaughter conditions or methods of capture, management and post-mortem treatment and storage time and temperature.

Texture is an important sensory attribute of fish and it has been measured by a diversity of sensorial and instrumental methods, with different results, as shown in a recent review paper (Hyldig and Nielsen, 2001).

In addition to the way of sensorial oral assessment, non-oral method are commonly performed in the fish sector, and consist of the press with a finger on the part of the fish body and observe how it behaves. They are very useful because they are fast, non-destructive and require little training. These non-oral tests have been used to define certain parameters, comprised on the quality index method (QIM) (Bremner et al., 1987). In many cases, the sensory analysis of texture is difficult to perform for reasons including time and cost (Szczseniak, 1987). So there has been an effort to design instrumental methods, correlated with both sensory and non-oral method.

Besides sensory methods, various techniques have been developed to measure fish texture and many information is available on the measurement of the texture of raw fish.

The dynamometry describes the methods and the tools necessary to mechanically reproduce the conditions under which a product is to be in reality. The dynamometric test is often an imitative analysis, useful primarily to obtain objective results comparable with the sensory evaluations.

This kind of measurements are performed with devices called dynamometers, able to evaluate the resistance that the food objects to the application of shear, compression, traction, extrusion, adhesion, etc.

Many instrumental methods have been developed to measure the textural properties of food (Bourne, 2002) and fish (Barroso et al., 1997; Hyldig and Nielson, 2001). Rheological analyses can been classified in three groups (Bourne, 2002):

• fundamental, when the rheological properties measured are well defined;

• empirical, when instrumental parameters are correlated with texture measured by sensory tests;

• imitative, which are those tests that resemble the conditions to which the food material is subjected in practice.

Most of the reported data for the evaluation of the quality of fish are based on mechanical tests that are empirical or imitative.

The fish industry has shown interest in the development of quick and not expensive non-destructive tests to evaluate the textural properties of fish and fish products. Recently attempts have been made to design new rheological methods both with large instrumental or portable instruments (Careche and Barroso, 2009).

Compression and puncture tests have been applied by Borderias et al., (1983) to both raw and cooked fillet and mince samples of five different fish species.

Good correlations between sensory hardness and chewiness of cooked fish samples, from 18 species from the families *Lufjanidae* and *Scorpaenidae,* and the maximum shear stress, obtained by puncture test have been reported (Sawyer et al., 1984). Botta (1991) developed a texture index derived from a compression test applicable to fillets of raw Atlantic cod (*Gadus morhua*), in particular this index was the ratio between the rebound distance and the deformation distance.

Orban and co-workers (1997) compared the texture of fish from intensive and extensive farming systems; the researchers found differences in the sensory texture of cooked fish, both in compression and puncture tests carried out on raw fish, but did not establish a correlation between them.

6.3.1 Difficulty in texture measuring

Within the same batch of fish, there may be considerable variation from fish to fish, and the properties of texture may vary along the location of the fish, thus adding difficulty to the measurement. For all fish, compression properties are influenced by the presence of the layer of skin. In this sense, the measures of the structure through the skin may be more robust as the skin acts as an envelope and provides a degree of spatial averaging. The blocks have complex structure of muscle fibers arranged in bundles actomyosin. A large percentage (about 80%) of muscle is water held by capillary forces between the bundles (Nesvadba 2010).

The compression of the muscle leads to hydrodynamic effects of water displacement. The beams are further assembled into blocks inclined (myotomes) typically measuring several millimeters and held together by connecting the fabric. Therefore, except for very homogeneous muscles, as the adductor muscle in combs, it is generally very difficult to excise from a fish sample a uniform fiber orientation and a reasonably isotropic structure. Moreover, the structure is inhomogeneous on medium scale (centimeters) due to the presence of bones of the skeleton (Nesvadba 2010).

On the scale comparable with the size of the fish, the presence of bodies of fish and the irregular geometry of the fish body can influence a compression test. These difficulties are common to all logical biomaterials, from vegetable and animal kingdoms. With the rapid advances in measurement technology, it is possible to look forward to solve the problems of so far intractable structure for texture characterization. Despite these

difficulties, the value of the measures is instrumental in quantitative determination of the objective of a number of mechanical properties.

Secondly, the interpretation of the results in terms of mechanical models allows an understanding of the mechanisms of deformation and fracture of the muscle fibers.
Such understanding and insight can then forecast to more theoretical base that is possible from the empirical sensory evaluations (Nesvadba 2010).

6.3.2 Stress Relaxation Test

Stress relaxation test is widely used in the rheological characterization of food products. It is an easy to perform method, which requires little sample preparation and could be used for quality assessment.

In relaxation test, the sample is subjected to a deformation, and the force required to holding the deformation constant is measured as a function of time. This rheological test is able to provide different parameters, such as: relaxation time, viscous, elastic modulus, as well as the "degree of solidity" of the food. A problem associated with this test is how to analyze the relaxation curve to obtain the above parameters. The use of non-linear regression, of two or three terms is sufficient to achieve a good fit of the experimental values (Mohsenin, 1970b; Peleg, 1979).

The possibility of using the stress relaxation test for the development of a non-destructive method to monitor post-mortem changes in cod (*Gadus morhua*) (Herrero et al., 2004) and in frozen stored hake (*Merluccius capensis* and *M. paradoxus*) have been studied.

6.4 Others

Other instrumental methods may be used for freshness assessment of fish and as part of production process control such as:

- Proton nuclear magnetic resonance (1H-NMR)

Ability of fast control of the composition of fish flesh along chain of production would give the fish producers the advantage of more control over fish spoilage during storage and improving the quality of the final product.

Proton nuclear magnetic resonance (1H-NMR) spectroscopy, is a rapid technique that can provide information on a wide range of metabolites. Sacchi et al. (1993) presented the quantitation of n-3 polyunsaturated fatty acids in fish lipids by 1H-NMR.

Amino acids are key molecules in both autolysis and biological spoilage reactions, their observation may offer alternatives to the K-index and its variants to follow fish quality loss during storage. Proton nuclear magnetic resonance (1H-NMR) can be in turn a suitable technique for this purpose, as suggested by **Paper III** and **Paper IV**.

- Dielectric properties assessment

During storage post-mortem changes in the fish muscle affect quality through mechanisms that could also alter the dielectric proprieties. Most obvious are changes affecting interactions between waters and proteins, and degradation processes leading to the formation of polar compounds (Kent and Jörg, 2009).

Measurements of dielectric properties have been tested and used during almost 40 years for quality grading and remaining shelf life determination of various fish. The Intellectron Fishtester, the Torrymeter and the RT-Freshtester represent instruments with increasing degrees of sophistication. Readings from all instruments reflect dielectrical properties of fish and they decrease with storage time, almost following a straight line. Based on these rapid and non-destructive measurements, the RT-Freshtester allows automatic grading of 60-70 fish per minute (seafood.ucdavis.edu/pubs/qualitysafety.doc).

Electrical properties of fish are not directly responsible for sensory spoilage and it is, therefore, to be expected that numerous factors influence the relationship between such measurements and seafood spoilage. In fact, these instruments need calibration depending on the season and fish handling procedures, and they are unsuitable for grading frozen/thawed fish, partially frozen (i.e. superchilled fish), fish chilled in refrigerated seawater, or for fish fillets. This and the high cost of the instruments limits their practical use in the seafood sector for freshness evaluation (seafood.ucdavis.edu/pubs/qualitysafety.doc)

- Near infra-red (NIR)

During the past years, the use of NIR spectroscopy has gained importance in the evaluation of food quality parameters (Osborne and Fearn, 1986; De Boever et al., 1992; Thyholt and Isaksson, 1997; Thybo et al., 2000). NIR has been used in fish for the determination of free fatty acids in mackerel (Zhang and Lee, 1997), fat, water and protein content in salmon and halibut (Downey, 1995; Wold and Isaksson, 1997; Nortvedt et al., 1998). This technology is useful also for measurement of water-holding capacity of frozen and thawed cod (Jørgensen and Jensen, 1997).

- Electronic noses

The composition and concentration of the volatile compounds emanating from fish depend on its freshness. Odour is an important sensory attribute to evaluate the quality of fish. Electronic noses are attempts to reproduce the functions of the natural olfaction sense that have been introduced as rapid assessment technique in food industries (Bartlett et al., 1997). Gas sensors or “electronic noses” have been employed for the rapid detection of volatile compounds formed by the degradation of food composition, as indicators of freshness or quality (Olafsdóttir et al., 1988).

7. The Adriatic Sea

The Adriatic Sea is a semi-enclosed sea, bordered in the southwest by the Apennine or Italian Peninsula, in the northwest by the Italian regions of Veneto and Friuli-Venezia Giulia, and in the northeast by Slovenia, Croatia, Bosnia-Herzegovina, Montenegro, and Albania, the Balkan peninsula. In the southeast, the Adriatic Sea connects to the Ionian Sea at the 72-kilometre (45 mi) wide Strait of Otranto. It extends 800 kilometers from the northwest to the southeast and is 200 kilometers wide.

It covers 138,600 square kilometers and has a volume of 35,000 cubic kilometers. The Adriatic extends northwest from 40° to 45°47' north, representing the Mediterranean's northernmost portion. The sea is geographically divided into the Northern Adriatic, Central (or Middle) Adriatic, and Southern Adriatic.

The chemical and physical characteristics of Adriatic sea water are strongly affected and influenced, by the water supply of the great river Po. The river Po along with other rivers which discharge into the Adriatic, increasing not only the amount of water, but they are also sources of nutrients for all of the organisms of this sea. Productivity in the Adriatic Sea is primarily due to the reduced depth of the sea. It is rich in flora and fauna and, and on it is possible to find a variety of caves, archaeological sites, shipwrecks and rich variety of marine life.

The Adriatic Sea (**Figure 7.1**) is inhabited by various organisms such as gastropods, bivalves, corals, fish, echinoderms, sponges, crabs, turtles, mammals such as dolphins, whales, and different types of algae etc. The differences in temperature and salinity (density), which established between the waters of the northern and southern

regions of the reservoir, determine a cyclonic circuit of current stable over time, ensuring the replacement of the general Adriatic waters.

The northern Adriatic circuit is divided in small local vortices (in the Gulf of Trieste, north of the Po, south of the Po and off south of the Po in the coastal zone) and is characterized by the presence of species that are live generally in these areas, such as whiting and sprats.

The circuits have effects on the distribution of species and populations, so that you can distinguish populations or localized stock mainly in the northern basin, mainly in the middle or in the south. In every part also occur the breeding areas and nursery areas frequented at specific times of the year. Each circuit hydrological is maintained separately from the other and stable over time, and therefore also each stock maintains its individuality, remains strongly correlated to the dynamics of the circuits.

Figure 7.1. Location where the fish species used for this research study (red mullet, bogue and european hake) have been fished

7.1 Fish catching systems

Sea fishing is any activity designed to capture specimens of species whose usual environment or natural life are the marine waters.

The capture of marine organisms can be carried out using different fishing systems (**Figure 7.2**). Among them, the most important are seine nets, seine nets, dredges, traps, hooks and lines, falling gears, gillnets and entangling nets, traps, gillnets, lift nets and trawling.

In Adriatic according to vessel size, professional fishing can be divided into:

1 - Large vessels engaged trawling and pair trawls;

2 - Small boats fishing with gillnet, traps (basket, pot, barrel or cage), fishing with hooks and also engaged trawling.

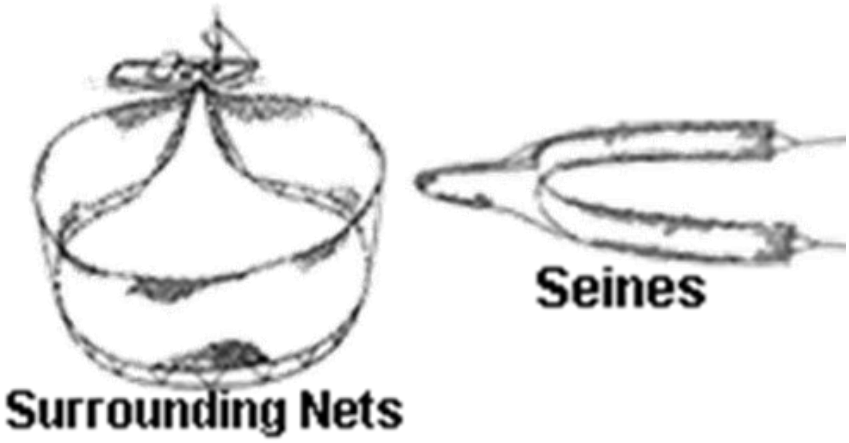
Trawl nets means nets which are actively towed by the main boat engine and consisting of a cone- or pyramid-shaped body (as trawl body) closed at the back by a cod-end, and which can extend at the opening by the wings or can be mounted on a rigid frame. Horizontal opening is either obtained by otter boards or provided by a beam or frame of variable shape and size. Such nets can be towed either on the bottom, “bottom trawl net” or in midwater, “pelagic trawl net” (Regulation (EC) No. 1967/2006). Red mullet examined in **Paper I** have been fished by pelagic trawl net.

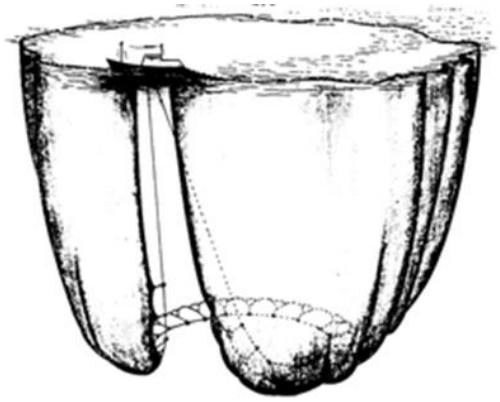
Pair trawls is practiced fishing for bluefish especially in Adriatic Sea and uses a pair of fishing vessels, that drag a network very big bag-shaped terminal at a certain height of the water column.

Fishing with gillnets is very widespread in Italy. It uses the so-called trammel to catch squid, mullet, scorpion fish, bream, sea bream, sea bass and lagoon species. Bogue examined in **Paper IV** have been fished in this way.

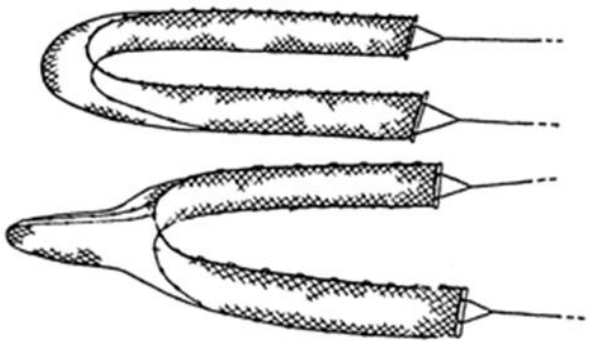
Traps are fishing gears which are fixed to or deployed on the bottom, and which acts as at rap to catch marine species. They are constructed in the form of a basket, pot, barrel or cage, and in the majority of cases they comprise a rigid or semi-rigid frame made of various material (wood, wicker, metal rods, wire netting, etc.) that may or may not be covered with netting. They have one or more funnels or mouths with smooth ends that allow species to enter the internal chamber. They may be used separately or in groups. When used in groups a main line carries numerous traps on branch lines of variable length and spacing, depending on the target species (Regulation (EC) No. 1967/2006). In this way (fishing with gillnets) species as cobs, octopus, squid, shrimp are captured.

Hook is a bent, sharpened piece of steel wire usually with barb. The point of a hook may be either straight or even reversed and curved; the shank can be of varying length and form and its cross section can be round (regular) or flattened (forged). The total length of a hook shall be measured as the maximum overall length of the shank from the tip of the hook, which serves for fastening the line and is usually shaped as an eye, to the apex of the bend. The width of a hook shall be measured as the greatest horizontal distance from the external part of the shank to the external part of the barb (Regulation (EC) No. 1967/2006).





Purse seines



Beach seines

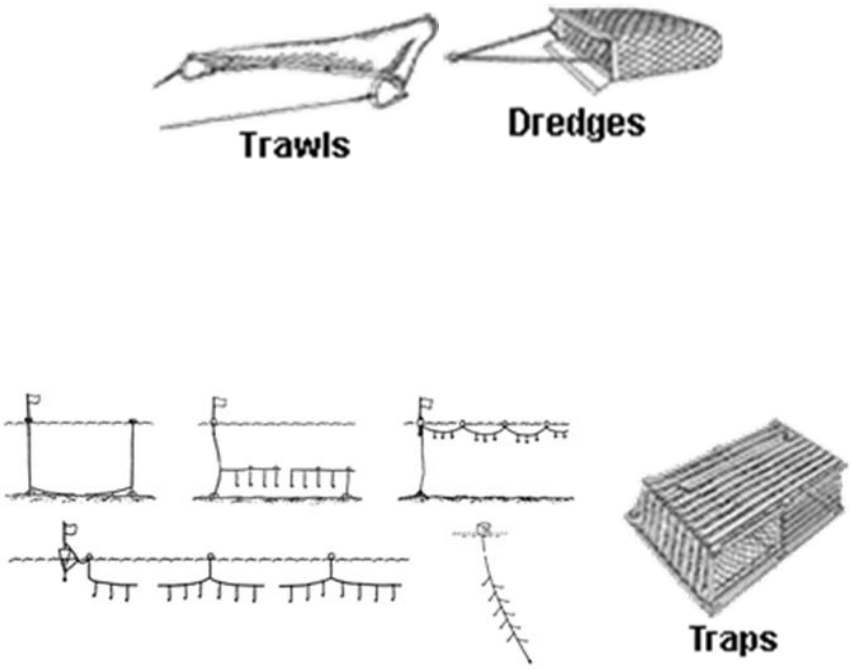


Figure 7.2. Illustration of fishing gear type act sheets (FAO, 2013)

7.2 Fish species in the Adriatic Sea

Table 7.1 lists the fish generally caught in the Adriatic Sea named in English, Italian and Latin.

English	***Italian***	**Latin**
Anchovy	Alice	*Engraulis encrasicholus*
Anglerfish	Rana pescatrice	*Lophius piscatorius*
Atlantic bonito	Palamita	*Sarda sarda*
Bogue	Boga	*Boops boops*
Big-scale sandmelt	Latterino	*Antherina mochon*
Blue whiting	Potassolo	*Micromesistius poutassou*
Cackerel	Mendola	*Maena maena*
Cod	Merluzzo	*Gadus*
Dentex	Dentice	*Dentex dentex*
Dogfish	Palombo	*Mustelus mustelus*
Eel	Anguilla	*Amguilla anguilla*
Goby	Ghiozzo	*Gobius*
Grouper	Cernia	*Ephinepelus guaza*
Gurnard	Capone	*Eutrigla gurnadus*
Herring	Sarda	*Clupea harengus*
Horse mackerel	Sugarello, Suro	*Trachurus trachurus*
Jack	Leccia	*Lichia amia*
Mackerel	Sgombro	*Scomber scombrus*
Mullet	Cefalo	*Chelon labrosus*
Mullet spp.	Triglia	*Mullus spp.*
Needlefish	Aguglia	*Belone belone*
Ox-eye	Orata	*Sparus aurata*
Ray	Razza	*Raja*
Sea bream	Pagello	*Pagellus bogaraveo*
Sole	Sogliola	*Solea vulgaris*
Sea bass	Spigola	*Dicentrarchus labrax*
Sword fish	Pesce spada	*Xiphias gladus*
Tuna	Tonno	*Thunnus thynnus*
Turbot	Rombo	*Psetta maxima*
Umbrine	Ombrina	*Umbrina cirrosa*
White bream	Sarago	*Diplodus*
Whiting	Merlano	*Merlangius merlangus*

Table. 7.1. English, Italian and Latin name of fish species

Ismea (Institute of Food Services for the Agricultural Market-Ismea - The fishing industry in Italy, 2010 check -up) confirmed that the species most caught by the Italian fleet in 2009 are anchovies (*Engraulis encrasicolus*), that have accounted for 23% of the

catch. Veneto, Emilia Romagna, Sicily, Puglia and Abruzzo, who offered the highest contribution to the total catch of anchovies (together covered 82%) see this species also in at the first place of the regional production. The anchovies and sardines are caught usually in massive quantities in the north and south Adriatic. In the first places for caught quantities, there are also sardine (*Clupea pilchardus*), hake (*Merluccius merluccius*)and mullet spp. (*Mullus barbatus and Mullus surmuletus*).

Other species of fish caught in a great quantity but only in specific months are Horse mackerel (*Trachurus trachurus*), whiting (*Merlangius merlangus*), and cod (*Gadus morhua*) and sole (*Solea solea*).

7.2.1 Hake (*Merluccius merluccius*)

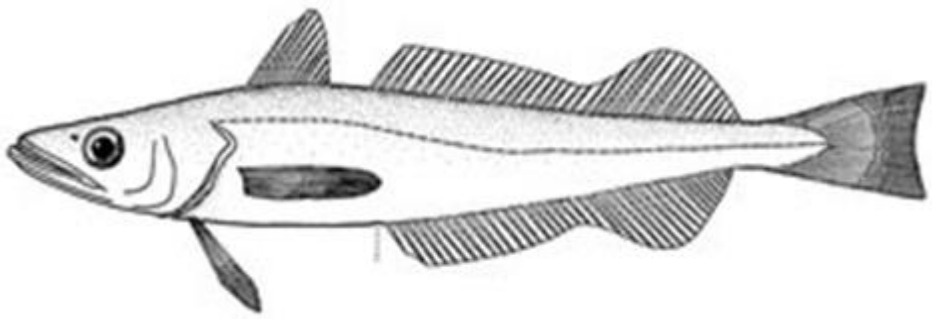


Figure 7.3.*Merluccius merluccius*
(Linneaus, 1758)

Kingdom: Animalia
Phylum: Chordata
Class: Actinopterygii
Order: Gadiformes
Family: Merlucciidae
Subfamily: Merlucciinae
Genus: Merluccius
Species: M. merluccius
Italian common name: Nasello
English common name: European hake

Morphology

European hake (*Merluccius merluccius*) has an elongated body and slender, moderately compressed at the sides and covered with small scales. It has a broad head, long and depressed in dorsal-ventral point. The wide mouth and prominent has two or three series of sharp teeth, of which the outer series are fixed short and sharp, while the internal ones are longer and valuable backward (Bini, 1968-70). In adults, the oral cavity and gill rooms are purplish black. The eye is round and not very big.

The fins have no spines. The anterior dorsal fin is short, high and sub-triangular, while the second is long and reaches to the caudal peduncle, but rarely observed intact. The height of the second dorsal decreases up to a certain point, and then increased up to have the rear higher from front.

The anal fin and symmetrical with respect to the previous one and is similar in shape and size. The caudal fin is slightly concave. The ventral jugular position, have similar dimensions to the pectorals. The colour is the typical fish backdrop, gray back with silvery sides and lighter, with silver highlights and a white belly.

Distribution and Habitat

European hake (*Merluccius merluccius*) is found in the Mediterranean Sea, the North Sea and the eastern Atlantic Ocean between Iceland and Mauritania. It is a night predator which during the day stays on sandy or muddy abyssal plains, at depths usually between 30 and 400 m, although it has been found at depths up to 1,000 meters. It can reach a length of about 140 cm, with a weight up to 15 kg (**Figure 7.4**).

The young feed on crustaceans, but as they grow they start to feed on small and medium-sized fish and cephalopods. The adults show cannibalistic behaviour, eating smaller members of their own species.

In Mediterranean have a size between 30 and 40 cm, but can also reach the 110 cm (140 cm and 15 kg, in the Atlantic).

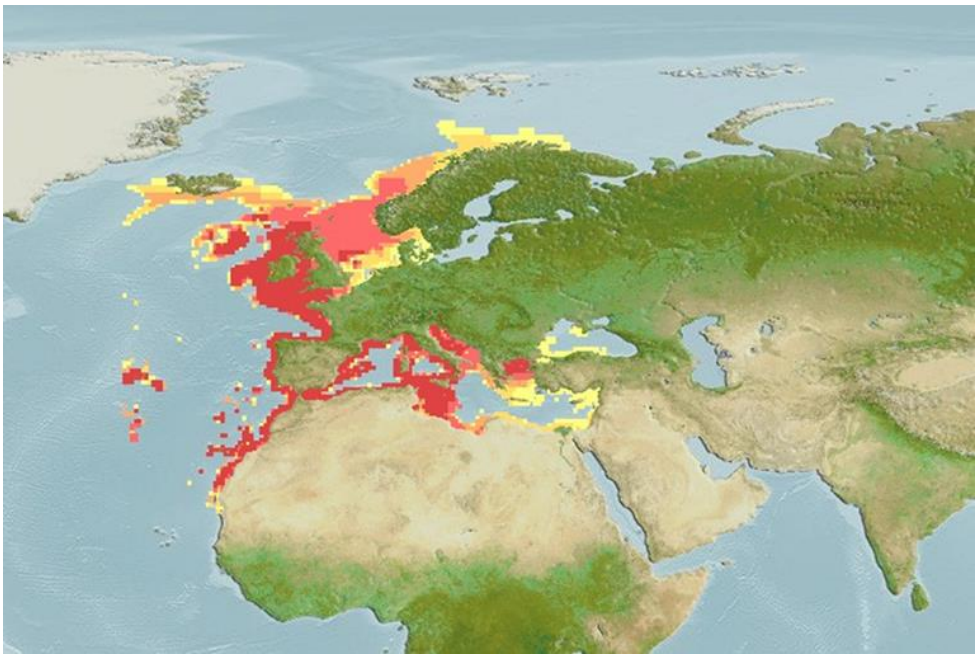


Figure 7.4. Geographical distribution of european hake (*Merluccius merluccius*) (FISHBASE, 2013)

These fishes live on sandy and muddy. Adults are common at depths between 70 and 370 m, but were also found individuals from 30 to 1000 meters, while the larval and youth living near the coast, without any connection to the bottom.

In the winter, migrates to deeper waters while in the summer season is to shallower depths. During the day, adults leave the bottom to feed.

In Italy the major fishing areas are the sea of Sicily, the middle and lower Adriatic and the Tyrrhenian Sea (Cataudella et al., 2000).The total catch reported for this species is reported in **Figure 7.5.** In the Mediterranean, the minimum landing size for this species according to Regulation (EU) No. 1976/2006 is 20 cm.

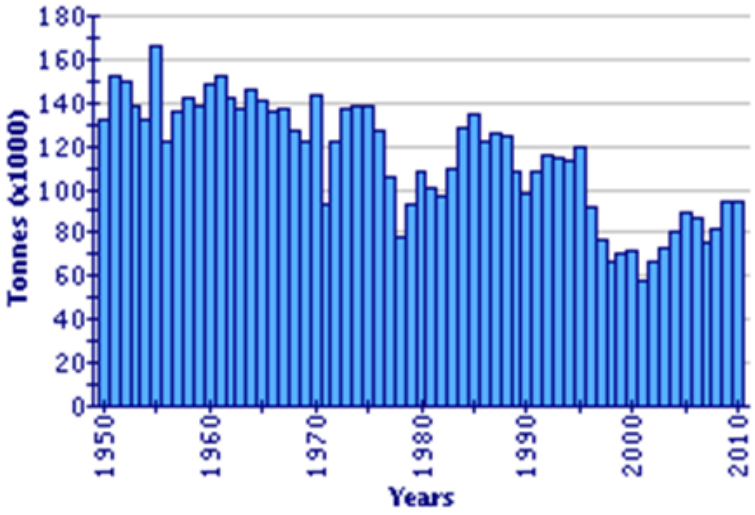


Figure 7.5. Global capture production for European hake (*Merluccius merluccius)* (FAO, Fishery Statistic)

Reproduction

The reproduction takes place during the entire period of the year, with a peak in winter in areas well-defined and in any case always in deep water (between 100 and 300 meters)(Fischer et al., 1987).

Eggs, from 2 to 7 million for female, are spherical, with a diameter approximately of 1 mm, and floating (equipped with a drop oily). During the breeding season, adults form large herds, and eventually dispersed in reproduction occurred. The larval and young *Merluccius merluccius* living near the coast, without any connection to the bottom.

Fishing

European hake (*Merluccius merluccius*) fishing in Italy generally is carried out with trawls, either on the bottom, ”bottom trawl net” or in midwater, “pelagic trawl net” (bottomwater end midwater trawl net), gillnet systems and with passive gear.

7.2.2 Red mullet (*Mullus barbatus*)

Figure 7.6. *Mullus barbatus*
(Linnaeus, 1758)

Classification
Kingdom: Animalia
Phylum: Chordata
Class: Actinopterygii
Order: Perciformes
Family: Mullidae
Genus: Mullus barbatus
Species: M. barbatus
Italian common name: Triglia
English common name: Red Mullet

Morphology

The genus Mullus is represented by two species that, red mullet (*Mullus barbatus*) and surmullets (*Mullus surmuletus*).

The main character of the species belonging to the genus Mullus is the presence of two long barbells, place the mandible, with sensory functions, and used to flush out the prey.

Red Mullet (Mullus Barbatus) has an elongated body and moderately compressed laterally. The head large enough relative to the body, the mouth is small and little protractile, opens horizontally at the bottom. The eye is located in the upper part of the head (Tortonese, 1975).

Red Mullet (*Mullus Barbatus*) has two dorsal fins well separated and the court, the first consisting of 7:00 to 8:00 spines, the second from 8:00 to 9:00 beams piers. The anal fin is located ventrally in correspondence of the second ridge and has a similar size. The caudal fin is forked with lobes of equal size. Finally, the pectoral fins are well developed and, below these, there are the ventral.

The body is covered by cast large that break off very easily, the colour is pink with red spots and there are often some longitudinal yellow stripes. *Mullus barbatus* can reach a size of 30 cm although rarely exceed 20 cm (Soljan, 1975; Fisher et al., 1987).

Distribution and habitat

This species is widespread in the Black Sea, the Mediterranean and the Atlantic Ocean, from Scandinavia to the Azores. It lives in sand, mud and gravel up to 300-500 meters deep and carries a group life (**Figure 7.7**).

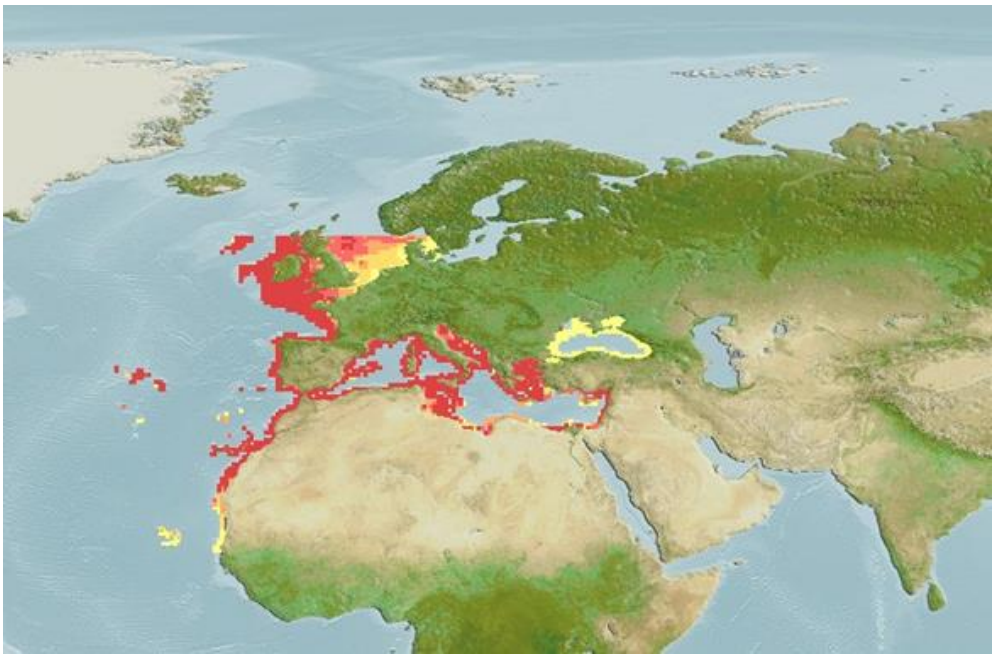


Figure 7.7. Geographical distribution of Red Mullet (*Mullus barbatus*) (FISHBASE, 2013)

The total catch reported for this species is reported in **Figure 7.8**. The mullet is fished throughout the year, with very pronounced peak in late summer-early fall. This time of year, young mullets make the migration-off cost to join the adult population (recruitment). In this period the activity of trawlers are able to catch the fish in massive

quantities. In the Mediterranean, the minimum landing size for the species of the genus Mullus according to Regulation (EU) No. 1976/2006 is 11 cm.

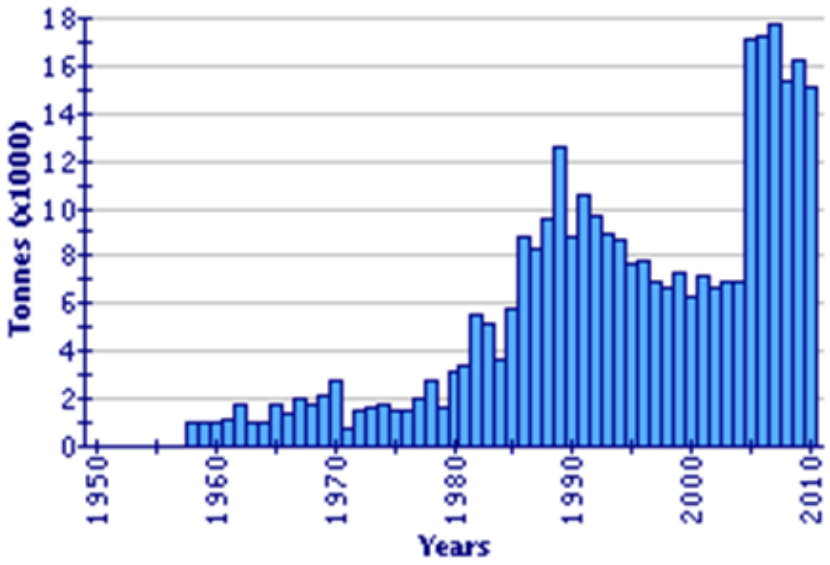


Figure 7.8. Global Capture production for Red Mullet (*Mullus barbatus*) (FAO, Fishery Statistic)

Reproduction

Red Mullet (*Mullus barbatus*) reproduces from April to July. The eggs become larvae, larval, post-larval and juvenile up to 4 cm in total length. In this period red mullet lead a pelagic life and have a blue colour. In the following period it tends to move towards the coast, staying longer on the seabed and prioritizing areas with brackish water. At the end of the summer, *Mullus barbatus* migrates from the coast to the deep, to join the adults. Also during this period of life it is typical that the livery blue becomes pink-red.

Fishing

Mullus barbatus is an important species for the quantities landed and caught mainly with gillnets, trammel nets and bottoms trawls. This species in the Adriatic is fished generally with bottom trawls and in some areas with Gillnets.

7.2.3 Bogue (*Boops boops*)

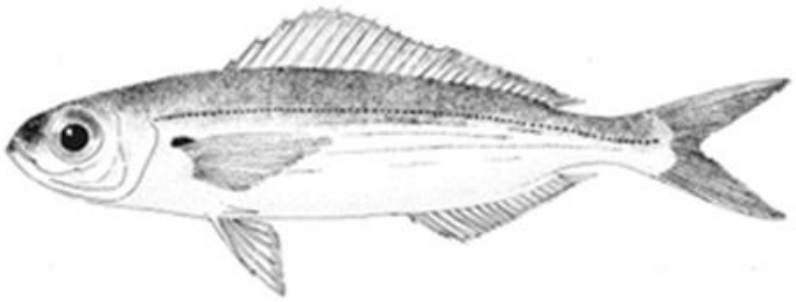


Figure 7.9. *Boops boops* (Linnaeus, 1758)

Classification:
Kingdom: Animalia
Phylum: Chordata
Class: Actinopterygii
Order: Perciformes
Family: Sparidae
Genus: Boops
Italian common name: Boga
English common name: Bogue

Morphology

Bogue (*Boops boops*) is a fish with a slender body, short snout and large eyes, the mouth is small and oblique, with a series of sharp teeth.

The back is olive green, the lateral line is dark brown and on the sides of the body, it is possible to see four yellow stripes; a small black spot is on top of the base of the pectoral fins and the fins are white.

Bogue (*Boops boops*) is a gregarious species from the semi-pelagic behaviour, feeding on crustaceans, algae and small fish.

The body of this fish is elongate, slender, with a short snout and large eyes, is covered with scales robust and relatively large; it can reach lengths that do not go beyond 35 cm. The head is conical with very large eyes and mouth has a small and oblique terminal, with thin and equal lips and small teeth arranged in a single row on both jaws.

The colour is greenish yellow with a metallic sheen on the back, silver sides, which fade more in the ventral part. On the body there are 3-4 horizontal stripes yellow-gold, much more evident when the fish is alive, while on the pectoral fins there is a small black spot.

Distribution and habitat

Bogue (*Boops boops*) is a demersal fish that lives near to the bottom gathers in flocks in sandy areas along the rocky coast.

It is common in the Mediterranean, Adriatic, Black Sea, Eastern Atlantic and the English Channel (**Figure 7.10**). During the night, it lives near the surface, while during the day remains closer to the bottom. In the Italian seas does not go beyond the 250 m depth.

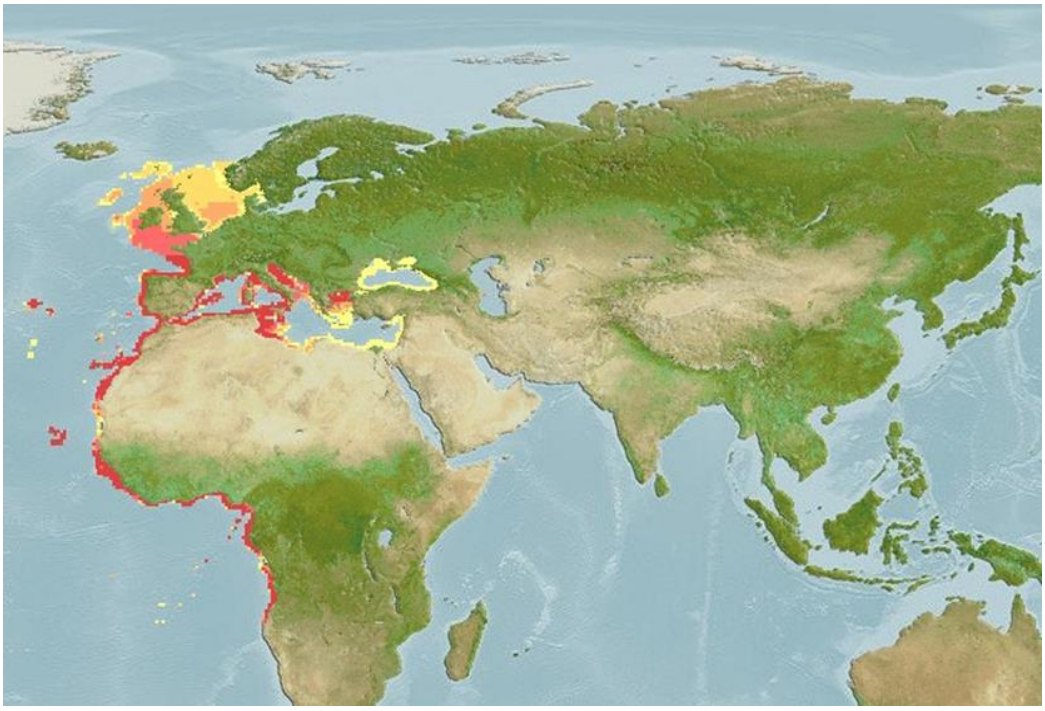


Figure 7.10. Geographical distribution of Bogue (*Boops boops*) (FISHBASE, 2013)

In Sicily, the size is slightly larger and are captured also with hook on founds of over 30 meters. It still considered a by-catch of commercial fishing to the low economic value. This fish has firm flesh and often they are used as bait for tuna fishing.

Bogue (Boops boops) is caught throughout the year although the best time is the summer season, from May to August. The total catch for this species are reported in **Figure 11**. This specie is one of the many species known as "poor fish" especially in Italy,

although it is very common, inexpensive and quite nutritious. For this is also the protagonist in times of initiatives and projects that take into account the protection of biodiversity.

Figure 7.11. Global Capture production for Bogue (*Boops boops*)
(FAO, Fishery Statistic)

Reproduction

Bogue (*Boops boops*) is a hermaphrodite species: all individuals are born female and aging become males. It breeds from February to April in the eastern Mediterranean and from April to May in the western Mediterranean, reaching maturity at about 12 cm in length. The eggs are buoyant and can be found in the plankton.

It is an omnivorous species, feeding on planktonic organisms, eggs, larvae and other marine organisms, especially fish. Bogue can reach at most the 36 cm in length but is common between 10 and 25 cm.

Fishing

Bogue (Boops boops) is caught on line gear, with bottom trawls, seine nets, purse seines and also with beach seines and trammel nets.

8. Conclusions

In general obtained results confirmed that the temperature of manipulation/conservation is a key factor in maintaining fish freshness, even if the response of the different species investigated to storage in ice and refrigeration was different.

NMR spectroscopy showed to be able to quantify and evaluate the kinetics for unselected compounds during fish degradation, even *a posteriori*. This can be suitable for the development of new parameters related to quality and freshness.

The development of physical methods, particularly the image analysis performed by computer vision system (CVS), for the evaluation of fish degradation, is very promising. Among CVS parameters, skin colour, presence and distribution of gill mucus, and eye shape modification evidenced a high sensibility for the estimation of fish quality loss, as a function of the adopted storage conditions. Particularly the eye concavity index detected on fish eye showed a high positive correlation with total QIM score.

Confirming previous findings this research shows that the development of physical methods for the evaluation of fish freshness presents great potentiality for the development of an instrumental quality index, correlated with both chemical and sensory indices, in order to lead to an integration and/or substitution of the standard sensory methods currently used.

It is important to underline that the different analytical techniques applied in this research for evaluation of fish freshness present advantages and disadvantages. The importance of combining more than one method of analyses is stressed, and the challenge is to find a rapid/integrated instrumental test to apply along the overall fish production chain.

REFERENCES

Ababouch, L. (2005) Composition of fish. In: FAO Fisheries and Aquaculture Department. Rome, Italy. Available at: http://www.fao.org/fishery/topic/12318/ en.

Ackman R.G. (1995) Composition and nutritional value of fish and shellfish lipids. In: Fish and Fisheries Products. Composition, Nutritive Properties and Stability. Ruiter A. Ed. CAB International, Wallingford, UK, 117-156.

Ackman R.G. and McLeod, C. (1988) Total lipids and nutritionally important fatty acids of some Nova Scotia fish and shellfish food products. Can. Inst. Food Sci. Technol. J., 21, 390-8.

Adams M. R. and Moss, M. O. (2008) Food Microbiology, Third Edition. The Royal Society of Chemistry, Thomas Graham House, Science Park, Milton Road, Cambridge CB4 0WF,UK.

Alasalvar C., Taylor K.D.A., Oksuz A., Garthwaite T., Alexis M.N., Grigorakis K. (2001) Freshness assessment of cultured sea bream (Sparus aurata) by chemical, physical and sensory methods. Food Chemistry 72, 33-40.

Alverson, D. L. (1976) Fishing gear and methods. In: In: M. E. Stand by (ed.), Industrial Fishery Technology. Robert E. Krieger, New York, 45-64.

Amato Neto V., Amato JG., Amato VS. (2007) "Probable recognition of human anisakiasis in Brazil". Rev. Inst. Med. Trop. Sao Paulo 49 (4) 261–2. PMID 17823758.

Anderson DM., White AW. (1992) Marine biotoxins at the top of the food chain. Oceanus 35(3) 55–61.

Antonacopoulos., N. and Vyncke W. (1989) Determination of volatile basic nitrogen in fish: A third collaborative study by Western European Fish Technologists' Association (WEFTA). Z. Lebensm., Unters., Forsch. 189, 309–316.

Ashie, I.N.A., Smith J.P., Simpson B.K. (1996) Spoilage and shelflife extension of fresh fish and shellfish. Crit. Rev. Food Sci. Nutr. 36, 87–121.

Atmar R.L., M.K. Estes (2001) Diagnosis of non-cultivatable gastroenteritis viruses, the human calciviruses. Clin. Microbial. Rev, 14, 15–37.

Audicana M.T; Kennedy MW. (2008) Anisakis Simplex: From Obscure Infectious Worm to Inducer of Immune Hypersensitivity. Clinical Microbiology Reviews 21 (2), 360–379. doi: 10.1128/CMR. 00012-07. PMC 2292572. PMID 18400801.

Baardseth P., Skrede G., NaesT., Thomassen M.S., Iversen A., Kaaber L. (1988) A comparison of CIE 1976L * a * b * values obtained from two different instruments on several foods commodities. Journal of Food Science 53, 1737-1742.

Baden DG., Trainer VL., (1993) The mode and action of toxins and seafood poisoning. In: Algal Toxins in Seafood and Drinking Water (Falconer IR, ed). San Diego: Academic Press, 49–74.

Baixas-nogueras S., Bover-cid S., Veciana-nogués T., NunesM.I., Vidal-carou M.C. (2003) Development of a quality index method to evaluate freshness in Mediterranean hake (*Merluccius merluccius*). Journal of food science, 68, 1067–1071.

Bambace G., Lucchetti A., (2011) Elementi di biologia della pesca. Italia-Milano: Il Sole 24 Ore Ed agricole, ISBN 978-88-506-5370-6.

Barile L. E., Milla A. D., Reilly A., Villadsen A. (1985) Spoilage patterns of mackerel (Rastrelliger faudhni Matsui). 2. Mesophilic and psychrophilic spoilage. ASEAN Food J. 1, 121-126.

Baross J., Liston J., (1970) Occurrence of Vibrio parahaemolyticus and related hemolytic vibrios in marine environments of Washington state. Appl. Microbiol. 20, 179-186.

Barros-Velázqueza J., Gallardo José M., Calo P., Aubourg Santiago P. (2008) Enhanced quality and safety during on-board chilled storage of fish species captured in the Grand Sole North Atlantic fishing bank. Food Chemistry, 493–500

Barroso M., Careche M., Borderias A.J. (1997) Evaluation of fish freshness using mechanical methods. In: Methods to Determine the Freshness of Fish in Research and Industry. Olafsdottir, G., Luten, J.B., Dalgaard, P., Careche, M., Verrez-Bagnis, V., Martinsdóttir, E., and Heia, K., Eds. International Institute of Refrigeration: Paris, France, 355.

Bartlett HN., Elliot JM., Gardner JW. (1997) Electronic noses and their application in food industry. Food Technology 51(12), 44-48.

Bechmann IE., Jørgensen BM. (1998) Rapid assessment of quality parameters for frozen cod using near infrared spectroscopy. Lebensm.-Wiss u-Technol 31, 648-652.

Bekaert, K. (2006) Development of a quality index method scheme to evaluate freshness in tub gurnard (*Chelidonichthys lucernus*). Seafood research from fish to dish: quality, safety and processing of wild and farmed fish, 289-296

Berger SA., Marr JS. (2006) Human Parasitic Diseases Sourcebook. Jones and Bartlett Publishers: Sudbury, Massachusetts.

Bini, G. (1968-1970) Atlante dei pesci delle coste italiane. 1-10. Mondo sommerso, Roma.

Bogdanovic´ T., Simat V., Frka-Roi´c A., Markovi K. (2012) Development and application of quality index method scheme in a shelf-life study of wild and fish farm affected bogue (*Boops boops*), Journal of Food Science, 51- 58.

Borderias A.J., Lamua M., Tejada M. (1983) Texture analysis of fish fillets and minced fish by both sensory and instrumental methods. International Journal of Food Science & Technology, 18, 85-95.

Botta, J.R. (1991) Instrument for nondestructive texture measurement of raw Atlantic cod (*Gadus morhua*) fillets. J. Food Sci. 56, 962-964, 968.

Botta, J. R. (1995) Evaluation of seafood freshness quality. New York: VCH Publishers, 65–97.

Bourne, M.C. (2002) Food texture and viscosity: Concept and measurement. In: Food Texture and Viscosity: Concept and Measurement. Taylor, S.L., Ed. Academic Press: New York.

Bramstedt, F. (1962) Amino acid composition of fresh fish and influence of storage and processing. In Fish in Nutrition; Heen, E., Kreuzer, R., Eds.; Fishing News (Books) Ltd.: London, UK, 61–67.

Bremner, H.A. (1985)A convenient easy to use system for estimating the quality of chilled seafood. Proceedings of the fish processing conference, Ed. Scott, D. N. and Summers, C. Nelson. New Zealand. Fish Proc. Bull. 7, 59-73.

Bremner H.A., Olley J., Vail A.M.A. (1987) Estimating the time temperature effects by rapid systematic sensory methods. In: D.E. Kramer and J. Liston (Eds) Seafood quality determination. Elsevier, Amsterdam, 413-434.

Bremner, H.A. (2000) Toward practical definitions of quality for food science, Crit. Rev. Food Sci. Nutr. 40, 83–90.

Burns G. B., Ke P. J., Irvine B. B. (1985) Objective procedure for fish freshness evaluation based on nucleotide changes using a HPLC system. Canadian Technical Report of Fisheries and Aquatic Science, 1373, 35.

Bykowski P., Dutkiewicz D. (1996) Freshwater fish processing and equipment in small plants. FAO Fisheries Circulars. C905, Rome, Italy.

Campas M., Prieto-Simon B., Marty J.L. (2007) Biosensors to detect marine toxins: assessing seafood safety, Talanta 72, 884–895.

Capozzi F., Cremonini M.A. (2008) Nuclear magnetic resonance spectroscopy in food analysis. In Handbook of Food Analysis Instruments; Otles, S., Ed.; CRC Press: Boca Roton, FL, USA, 281–318.

Cappelli P., Vannucchi V. (2005) Chimica degli alimenti. Conservazione e Trasformazioni Zanichelli editore, Bologna.

Careche M., Barroso M. (2009) Instrumental texture measurement. In: Fishery Products: Quality, Safety and Authenticity. United Kingdom: Wiley-Blackwell publishers, 214-239.

Cataudella, S., Carrada, GC. (2000) Un mare di risorse. Introduzione alla conservazione e dalla gestione delle risorse ittiche.

Cianti L., Boccetti M., Pelatti E., Cellesi E., Catalano A., Perico A., Bavazzano P., Colzia., Gravina M.T., Boddi V. (2007) L'indice di freschezza del pesce: proposta di un nuovo metodo di valutazione. Ind. Alim., 46, 997-1003.

Clydesdale, F. M. (1991) Color perception and food quality. Journal of Food Quality 14, 61-74.

Connell, J. J. (1995) Control of fish quality.4th ed. London: Fishing News Books Limited.

Connor, W.E. (2000) Importance of n-3 fatty acids in health and disease. American Journal of Clinical Nutrition. 71 (1), 171-175.

Dalgaard, P. (2000a) Fresh and lightly preserved seafood. In Shelf-Life Evaluation of Foods. 2nd ed. Edited by Man CMD, Jones AA. London: Aspen Publishers, Inc. 110-139.

Dalgaard, P. (2000b) Freshness, quality and safety in seafoods. Flair-Flow Europe Technical Manual F-FE 380A/00.

Damoglou, A. P. (1979) Quality assesment. In: J. J. Connel et al., Advances in Fish Science and Technology. Proceedings of an Jubilee Conference of the Torry Research Station, Aberdeen, Scotland. July 1979. Fishing News books Ltd., Farnham, Surrey, England, 394-399.

De Boever JL., Cottyn BG., Fiems LO., Boucquè CV. (1992) Determination of chemical composition of beef meat by NIRS. In: Hildrum KI, Isaksson T, Naes T, Tandberg A, editors. Near infra-red spectroscopy. Bridging the gap between data analysis and NIR applications. Chichester, West Sussex: Ellis Harwood Series in Analytical Chemistry. 339-344.

Delbarre Ladrat C., Cheret R., Taylor R. and Verrez-Bagnis V. (2006) Trends in postmortem aging in fish: understanding of proteolysis and disorganization of the myofibrillar structure. Critical reviews in food science and nutrition 46, 409-421.

Di Natale C, (2003) Data fusion in MUSTEC: towards the definition of an artificial quality index. Index in Luten, J. B., Oehlenschläger, J., Ólafsdóttir, G. (Eds). Quality of Fish from Catch to Consumer: Labelling, Monitoring and Traceability. Wageningen Academic Publisher, 273-382.

Donn R. W., Hackney C. (1991) Microbiology of Marine Food Products. AVI Book, Van Nostrand Reinhold, Cameron R.(Edit), New York (USA).

Downham A., Collins P. (2000) Coloring our foods in the last and next millennium. International Journal of Food Science Technology 35, 5-22.

Dunajski, E. (1980) Texture of fish muscle. Journal of Texture Studies, 10, 301–318.

Ehira S., Uchiyama, H. (1987) Determination of fish freshness using the K value and comments on some other biochemical changes in relation to freshness. In D. E. Kramer &J. Liston (Eds.), Seafood quality determination. Amsterdam: Elsevier Science Publishers, 185-207.

FAO, (1973) Code of practice for fresh fish. FAO Fisheries Circ. C318. Food and Agriculture Organization of the United Nations, Rome, Italy.

FAO, (2004) Marine Biotoxins: Food and Nutrition Paper 80, Food and Agriculture Organization (FAO) Rome, Report.

Feldhusen F. (2000) The role of seafood in bacterial foodborne disease. Microbes and Infection 2, 1651-1660.

Fennema O.R. (1990) Comparative water holding properties of various muscle foods. A critical review relating to definitions, methods of measurement, governing factors, comparative data and mechanistic matters, Journal of Muscle Foods, 1, 363–381.

Fernández-Salguero J., Mackie I. (1987) Comparative rates of spoilage of fillets and whole fish during storage of haddock (Melanogrammus aeglefinus) and herring (*Clupea harengus*) as determined by the formation of non-volatile and volatile amines. International Journal of Food Science and Technology 22, 385–390.

Fischer W., Bauchot M.L., Schneider M. (1987) Fiches FAO d'identification des espécespour les besoins de la peche (Révision 1). Méditerranée et mer Noir. Zone de peche 37. 1. Vegetaux et Invertebres. Publication préparéepar la FAO (Project GCP/INT/422/EEC). Rome, FAO, 760.

Gill, T. (1990) Objective analysis of seafood quality. Food Reviews International 6(4) 681-714.

Gill T. (1995) Autolytic changes. In: Huss HH, eds. Quality and quality changes in fresh fish. Rome: FAO Tech Paper No. 348, 39-51.

Gillespie N. C., MacRae I. C., (1975) The bacterial flora of some Queenesland fish and its ability to cause spoilage. J. of Appl. Bacteriol. (39), 91-100.

Glass R.I., J. Noel T. Ando R. Fankhauser., Belliot G., Mounts, A., Parashar, U. D., Breese,. J. S., Monroe, S. S. (2000) The epidemiology of enteric calcino viruses from humans: A reassessments using new diagnostics. J Infect Dis 181 (2) 254-61.

Gorzyka E., PekPoh Len (1985) Mesophilic spoilage of bay trout (*Arripistrutta*), bream (*Acanthropagrus butcheri*) and mullet (*Aldrichettaforsterri*). Reilley A (Eds), FAO fish report (317) suppl. 474, 123-132.

Gram, L. (1990) Spoilage of three Senegalese fish species stored in ice and at ambient temperature. Paper presented at SEAFOOD 2000 in Halifax, Canada. 12-16 May 1990.

Gram L., Christina Wedell - Neergaard Huss H. H. (1990) The bacteriology of fresh and spoiling Lake Victoria Nile perch (*Lates niloticus*). Int. J. of food Microbiology. 10, 303-316.

Gram L., Huss H. H. (1996) Microbiological spoilage of fish and fish products. International Journal of Food Microbiology, 33, 121-137.

Gram L., Huss, H. H. (2000) Fresh and processed fish and shellfish. Irradiation. In: Lund, B. M., Baird-Parker, T. C. & Gould, G. W. (Eds.), The Microbiological Safety and Quality of Foods. Aspen Publishers, 472-506.

Gram L., Ravn L., Rasch M., Bruhn J.B., Christensen A.B., Givskov M. (2002) Food spoilage- interactions between food spoilage bacteria. International Journal of Food Microbiology 78, 79–97.

Handumrongkul C., Silva J.L. (1994) Aerobic counts, colour and adenine nucleotide changes in CO2 packed refrigerated striped bass strips. Journal of Food Science 59, 67-69.

Hatae K., Tobimatsu A., Takeyama M. Matsumoto J.J. (1986) Contribution of the connective tissues on the texture difference of various fish species. Bulletin of the Japanese Society of scientific Fisheries 52, 2001-2007.

Hatae K., Yoshimatsu F., Matsumoto J. J. (1990) Role of muscle fibers in contributing firmness and coke fish. Journal of food science 55, 693-696.

Hattula T., Kievaara M., Moran M. (1993) Freshness evaluation in European whitefish (Coregonus wartmanni) during chill storage. Journal of Food Science, 58, 1212-1215.

He K., Song Y., Daviglus M.L., Liu K., Van Horn L., Dyer A.R. Greenland P. (2004) Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies. Circulation. 109, 2705-2711.

Herrero A.M., Huidobro A., Careche M. (2003) Development of a quality index method for frozen Hake (M. capensis and M. paradoxus). Journal of food science, 68, 3, 1086-1092.

Herrero A.M., Heia K., M. (2004) Stress relaxation test for monitoring post mortem textural changes of ice–stored cod (Gadus morhua). Journal of Food Science 69, FEP178– FEP182.

Hobbs G., Hodgkiss W. (1982) The bacteriology of fish handling and processing. In: Davis, R. (ed.), Developments in Microbiology. Applied Science publishers, London, 71-117.

Howgate, P. (1977) Aspects of fish texture. In: G.G. Birch, J,G. Brennan and K.J. Parker (Eds) Sensory properties of food. Applied science Publishers, London, UK, 249-269.

Huidobro A., Pastor A., Tejada M. (2000) Quality index method developed for raw gilthead seabream (*Sparus aurata*). Journal of Food Science, 65, 7, 1202-1205.

Huidobro A., Pastor A., Lopez-Caballero M.E., Tejada M. (2001) Washing effect of the quality index method (QIM) developed for raw gilthead sea bream (Sparus aurata). European Food Research and Technology, 212, 4, 408-412.

Huss H., Dalsgaard D., Hansen L., Ladefoged H., Pedersen A., Zitan L. (1974) The influence of hygiene in catch handling on the storage life of iced cod and plaire. Journal of Food Technology, 9, 213-221.

Huss, H.H. (1995) Quality and quality changes in fresh fish, Rome: FAO Fisheries Technical Paper No. 348, 195.

Huss H.H., Dalgaard P., Gram L. (1997) Microbiology of fish and fish products, In: Luten, J.B., Borresen, T., Oehlenschlager, J. (Eds.), Proc. Int. Seafood Conf., Seafood from Producer to Consumer, Integrated Approach to Quality. Elsevier, Amsterdam, 38, 413-430.

Huss, H.H. (1988) Fresh fish-quality and quality changes. Series No. 29, FAO, Danish International Development Agency.

Hyldig G., Nielsen, J. (1998) A rapid sensory method for quality management. In Methods to determine the freshness of fish in research and industry, Paris: Institut International du Froid, 297–300.

Hyldig g., Nielsen., j. (2001) A review of sensory and instrumental methods to evaluate the texture of fish muscle. J. Texture Studies 32, 219-242.

Inácio P., Bernardo F., Vaz-Pires P. (2003) Effect of washing with tap and treated seawater on the quality of whole scad (trachurus trachurus). Eur food res technol 217, 406–411.

Jacober L. F., Rand A. G. J. (1982) Biochemical evaluation of seafood. In: R. E. Martin, G. J. A. Flick, G. E. Hebard., D. R. Ward (Eds.), Chemistry and biochemistry of marine food products. Westport: CT AVI, 347-366.

Jobling M., Johansen S.J.S., Foshaug H., Burkow I.C., Jorgensen E.H. (1998) Lipid dynamics in anadromous Arctic charr, Salvelinus-Alpinus (L.): seasonal variations in lipid storage depots and lipid class composition. Fish Physiol. Biochem., 18, 225-240.

Johnston I. A., Alderson R., Sandham, C., Dingwall A., Mitchell D., Selkirk C., Nickel D., Baker R., Robertson B., Whyte D., Springate J. (2000) Muscle fibre density in relation to the colour and texture of smocked Atlantic salmon (Salmosalar L.). Aquaculture189, 335-349.

Jørgensen BM., Jensen HS. (1997) Can near-infrared spectrometry be used to measure quality attributes in frozen cod? In: Luten JB, Borresen T, Oehlenschlager J, editors. Seafood from producer to consumer, Integrated approach to quality. Amsterdam: Elsevier Science, 491-496.

Karube I., Matsuoka H., Suzuki S., Watanabe E., Toyama K. (1984) Determination of fish freshness with an enzyme sensor system. Journal of Agricultural and Food Chemistry 32, 314-319.

Kent M., Jörg O. (2009) Measuring Electrical Properties in: Fishery Products: Quality, Safety and Authenticity. United Kingdom: Wiley-Blackwell publishers.

Koutsoumanis K., Nychas GE. (1999) Chemical and sensory changes associated with microbial flora of Mediterranean boque (*Boops boops*) stored aerobically at 0, 3, 7 and 10 °C. Applied Environmental Microbiology 65, 698-706.

Lara J.J., Economou M., Wallace A.M., Rumley A., Lowe G., Slater C., Caslake M., Sattar, N., Lean M. E.J. (2007) Benefits of salmon eating on traditional and novel vascular risk factors in young, non-obese healthy subjects. Atherosclerosis. 193, 213-221.

Lawless H.T., Heymann H. (1998) Sensory Evaluation of Food. Principals and Practices. Chapman& Hall, New York, 379-405.

Lees, D. (2000) Viruses and bivalve shellfish. Int J Food Microbial 59, 81–116.

Le Guyader F. S., Bon F., DeMedici D., Parnaudeau S., Bertone A., Crudeli S., Doyle A., Zidane M., Suffredini E., Kohli E., Maddalo F., Monini M., Gallay A., Pommepuy M., Pothier P., Ruggeri F. M. (2006) Detection of multiple noroviruses associated with an

international gastroenteritis outbreak linked to oyster consumption. J. Clin. Microbiol. 443878-82.

León K., Mery D., Pedreschi F., León J. (2006) Color Measurement in L*a*b* units from RGB digital images. Food Research International 39, 1084-1091.

Lima dos Santos, (1978) Bacteriological spoilage of Amazonian fresh water catfish (*Brachyplatistoma vaillanti valenciennes*). In Gram et al., 1990: The bacteriology of fresh and spoiling Lake Victoria Nile perch (*Lates niloticus*). Int. J. of food Microbiology. (10), 303-316.

Liston J., Stansby M. E., Olcott H. S. (1976) Bacteriological and chemical basis for deteriorative changes. In: Stansby M. E. (ed.). Industrial Fishery Technology. Robert E. Krieger, New York, 345-358.

Liston, J. (1980) Microbiology in fishery Science. In: J.J. Connell and staff of Torry Research Station (Eds). Advances in Fish Science and Technology., Fishing News Book Ltd., London, 138-157.

López-Caballero M., Huidobro A., Pastor A., Tejada, M. (2002) Microflora of gilthead seabream (*Sparus aurata*) stored in ice. Effect of washing. European Food Research and Technology, 215, 396-400.

Lund B.M., Baird-Parker A.C. and Gould G.W. (2000) The Microbiological Safety and Quality of Foods. Aspen Publishers, Inc. Gaithersburg, Maryland, USA, 1885 p.

Luong J. H. T., Male K. B. (1992) Development of a new bio -sensor system for the determination of the hypoxanthine ratio, an indicator of fish freshness. Enzyme and Microbial Technology 14, 125-130.

Luten J.B., Martinsdóttir E. (1997) QIM: a European tool for fish freshness evaluation in the fishery chain. In: Ólafsdóttir G, Luten J, Dalgaard P, Careche M, Verrez-Bagnis V, Martinsdóttir E, Heia K (eds.) Methods to determine the freshness of fish in research and industry. Proceedings of the Final Meeting of the Concerted Action "Evaluation of Fish Freshness", AIR3CT94 2283, Nantes Conference, November 12-14 1997. International Institute of Refrigeration, Paris, France, 287-296.

Lyhs U., Schelvis-Smit A.A.M. (2005) Development of a quality index method (QIM) for maatjes herring stored in air and under modified atmosphere. Journal of Aquatic Food Product Technology, 14 (2), 63 - 76.

Macagnanoa A., Careche M., Herrero A., Paolesse R., Martinelli E., Pennazza G., Carmona P., D'Amico A., Di Natale C. (2005) A model to predict fish quality from instrumental features. Science Direct, Sensors and Actuators B 111–112, 293–298.

MacDougall, D.B. (2002) Color in food: Improving quality. Wood head publishing Ltd., Abington, Cambridge, 378.

Martinsdóttir E., Sveinsdóttir K., Luten JB., Schelvis-Smit R., Hyldig G. (2001) Sensory evaluation of fish freshness. Reference manual for the fish sector. Ijmuiden: QIM-Eurofish, 58.

Miller A., III, Scanlan R. A., Lee J. S., Libbey L. M.(1973a) Identification of the volatile compounds produced in sterile fish muscle (*Sebastes melonops*) by Pseudomonas fragii. Appl. Microbiol. 25, 952-955.

Miller A., III, Scanlan R. A., Lee J. S., and Libbey L. M. (1973b) Volatile compounds produced in sterile fish muscle (*Sebaste smelonops*) by Pseudomonas putrefaciens, Pseudomonas fluorescens and an Achromobacter species. Appl. Microbiol. 26, 18-21.

Mohsenin, N.N. (1970) Some basic concepts of rheology. In N.N. Mohsenin (Ed)Physical Properties of Plant and Animal Materials, Volume 1 (Structure, Physical Characteristics, and Mechanical Properties). Gordon and Breach Science Inc., New York, 88-173.

Nesvadba, P. (2010) Quality control by instrumental texture measurements. In: Seafoods Technology, Quality and Nutraceutical Applications 2010 Springer, Berlin.

Nielsen J., Hansen T. K., Jonsdóttir S., Larsen E. P. (1992) Development of methods for quality index of fresh fish. FAR Meeting, Noordwijkerhout, Netherlands.

Nielsen, J. (1997) Sensory analysis in fish. In Ólafsdóttir, G., Luten, J., Dalgaard, P., Careche, M., Verrez-Bagnis, V., Martinsdóttir, E. and Heia, K. eds. Methods to determine the freshness of fish in research and industry. Proceedings of the Final Meeting of the Concerted Action ‘‘Evaluation of Fish Freshness’’, AIR3CT94 2283 (FAIR Programme of the EU) Nantes Conference, November 12–14. Paris: International Institute of Refrigeration, 279–286.

Nielsen D., Hyldig G. (2004) Influence of handling procedures and biological factors on the QIM evaluation of whole herring (*Clupea harengus*). Food Research International, 37, 975–983.

Nortvedt R., Torrissen OJ., Tuene S. (1998) Application of near-infrared transmittance spectroscopy in the determination of fat, protein and dry matter in Atlantic halibut fillet. Chemometrics and Intelligent Lab Systems. 42, 199-207.

Offer G., Knight P. (1988) The structural basis of water-holding in meat Part 1: General principles and water uptake in meat processing. In Developments in Meat Science; Lawrie, R., Ed.; Elsevier Science Publishers Ldt: Barking, Essex, UK, 4, 63-172.

Olafsdóttir G., Martinsdóttir E., Oehlenschläger J., Dalgaard P., Jensen B., Undeland I., Mackie I., Henehan G., Nielsen J., Nilsen H. (1997) Methods to evaluate fish freshness in research and industry. Trends in Food Science and Technology, 8, 258-265.

Olafsdóttir G., Luten J., Dalgaard P., Careche M., Verrez-Bagnis V., Martinsdóttir E., Heia K. (1998) Methods to Determine the Freshness of Fish in Research and Industry, Proceeding of the Final Meeting of the Concerted Action "Evaluation of Fish Freshness" AIR3CT94 2283. Paris: International Institute of the Refrigeration (Co-sponsorship and Publication), 396 p.

Olafsdóttir G., Nesvadba P., Di Natale C., Careche M., Oehlenschlager J., Tryggvadóttir S.V., Schubring R., Kroeger M., Heia K., Esaiassen M., Macagnano A., Jørgensen B.M. (2004) Multisensor for fish quality Determination. Trends in Food Science & Technology 15, 86–93.

Oehlenschläger J., & Sorensen N. K. (1998) Criteria of seafood freshness and quality aspects. In Methods to determine the freshness of fish in research and industry. Proceedings of the final meeting of the concerted action AIRCT94-2283, Nantes. Paris: Institut International du Froid, 30–35.

Orban E., Sinesio F., and Paoletti F. (1997) The functional properties of the proteins, texture and the sensory characteristics of frozen sea bream fillets (*Sparus aurata*) from different farming systems. Lebensm.-Wiss. u.- Technol. 30 , 214-217.

Osborne BG., Fearn T. (1986) Near infrared spectroscopy in food analysis. Essex: Longman Scientific & Technical. 200 p.

Osek J., Wieczorek K., Tatarczak M. (2006) Seafood as potential source of poisoning by marine biotoxins, Medycyna Wet 62, 370–373.

Özyurt G., Kuley E., Özkütük S., Özogul F. (2009) Sensory, microbiological and chemical assessment of the freshness of red mullet (*Mullus barbatus*) and goldband goatfish (*Upeneus moluccensis*) during storage in ice. Food Chemistry 114, 505–510.

Pacheco-Aguilar R., Lugo-Sanchez M. E., Robles-Burgueno M. R. (2000) Postmortem biochemical and functional characteristic of Monterey sardine muscle stored at 0°C. Journal of Food Science, 65, 40–47.

Pastoriza L., Sampedro G., Herrera JJ., Cabo M. (1998) Influence of sodium chloride and modified atmosphere packaging on microbiological, chemical and sensorial properties in ice storage of slices of hake (*Merluccius merluccius*). Food Chem 61 (1/2), 23-28.

Peleg, M. (1979) Characterization of stress relaxation curves of solid foods. Journal of Food Science 44, 277-281.

Pérez-Villarreal B., Howgate P. (1987) Spoilage of European hake (*Merluccius merluccius*). Journal of the Science of Food and Agriculture 41 (4), 335-50.

Pivarnik L.F., Kazantzis D., Karakoltsidis P.A., Constantinides S., Jhaveri S.N., Rand A.G. (1990) Freshness assessment of six New England fish species using the Torrymeter. J. Food Sci. 55 (1) 79–82.

Regulation (EC) No.2406/1996 of the Council of 26 November 1996laying down common marketing standards for certain fishery products. Official Journal L 334, 1-24.

Regulation (EC) No. 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Official Journal of the European Communities L 031, 1 – 24.

Regulation (EC) No. 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. Official Journal of the European Communities L 139, 1 - 54.

Regulation (EC) No. 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin. Official Journal L 139, 55 -205.

Regulation (EC) No. 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organization of official controls on products of animal origin intended for human consumption. Official Journal L 139, 206 -320.

Regulation (EC) No. 882/2004 of the European Parliament and of the Council of29 April 2004on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. Official Journal L 191, 1-61.

Regulation (EC) No.1967/2006 of the Council of 21 December 2006 concerning management measures for the sustainable exploitation of fishery resources in the Mediterranean Sea, amending Regulation (EEC) No 2847/93 and repealing Regulation (EC) No 1626/94. Official Journal L 409, 11-85.

Regulation (EC) No. 1224/2009 of the Council of 20 November 2009 establishing a Community control system for ensuring compliance with the rules of the common fisheries policy. Official Journal L 343, 1-50.

Ruxton C.H.S., Reed S.C., Simpson M.J.A. e Millington K.J., (2004) The health benefits of omega-3 polyunsatured fatty acids: a review of the evidence. J. Hum. Nutr. Dietet., 17, 449-459.

Ryser E. T., Marth E. H., Taylor S. L. (1984) Histamine production by psychrotrophic pseudomonads isolated from tuna fish. J. Food Prot. 47, 378-380.

Sacchi R., Medina T., Aubourg S.P., Addeo F., Palillo L. (1993) Proton Nuclear Magnetic Resonance Rapid and Structure-Specific Determination of ω-3 Polyunsaturated Fatty Acids in Fish Lipids, J. Am. Oil Chem. Soc. 70, 225–228.

Sato K., Yoshinaka R., Sato M., Shimizu Y. (1986) Collagen content in the muscle of fishes in association with their swimming movement and meat texture. Bulletin of the Japanese Society of scientific Fisheries 52, 1595-1600.

Sawyer F.M., Cardello A.V., Prell P.A. Johnson E.A., Segars R.A., Maller, 0., Kapsalis, J. (1984) Sensory and instrumental evaluation of snapper and rockfish species. J. Food Sci. 49, 727-733.

Schubring, R. (2010) Quality Assessment of Fish and Fishery Products by Color Measurement.In: Handbook of Seafood and Seafood Products Analysis. Taylor and Francis Group, LLC, 395-424.

Shahidi, F. (1995) Seafood proteins and preparation of proteins concentrates. In: Seafood chemistry, processing technology and quality, Shahidi F. and Botta J.R. Eds. Chapman & Hall, UK , 3-9.

Shahidi F., and Cadwallader, K.R. (1997) Flavor and lipid chemistry of seafoods: An overview. In Flavor and Lipid Chemistry of Seafoods (F. Shahidi and K.R. Cadwallader, eds.) American Chemical Society, Washington, DC, 1–8.

Shewan, JM. (1949) Some bacteriological aspects of handling, processing and distribution of fish. J R Sanit Inst. 59, 394–402.

Shewan, J. M. (1962) The bacteriological of fish and spoiling fish and some related chemical changes. Recent adv. Food Sci. (1) 167-193.

Shewan, J. M. (1971) The microbiology of fish and fishery products – A progress report. J. Appl. Bacterial. 34, 299-315.

Shewan, J. M. (1977) The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. In Proceedings of the Conference on Handling, Processing and Marketing of Tropical Fish. London: Tropical Products Institute ,51-66.

Shuttle, C.A. (2007) Marine viruses major players in the global ecosystem. Nat rev. microbial 5, 801-12.

Soljan, T. (1975) I pesci dell'Adriatico. Mondatoried, 522.

Stien L.H., Amundsen A.H., Mørkøre T., Okland S.N., Nortvedt R. (2006a) Instrumental color analysis of Athlantic Salmon (*Salmo salar* L.) muscle. In: J.B. Luten, C. Jacobson, K Bekaert, A Saebo and J. Oehlenschlager (Eds). Seafood research from fish to dish. Quality, Safety and Processing of Wiled and Farmed Fish. Wageningen Academic Publishers, Wageningen, 525-539.

Stien L.H., Manne F., Ruohonen K., Kause A., Rungruangsak-Torrissen K., Kiessling A. (2006b) Automated image analysis as a tool to quantify the color and composition of rainbow trout (*Oncorhynchus mykiss* W.) cutlets. Aquaculture 261-705.

Sveinsdóttir K., Hyldig G., Martinsdottir E., Jørgensen B., Kristbergsson K. (2003) Quality index method (QIM) scheme developed for farmed atlantic salmon (*Salmosalar*). Food quality and preference 14, 237–245

Szczesniak, A.S. (1963) Classification of textural characteristics. Journal of food science 28, 385-389.

Szczseniak, A.S.(1987) Correlating sensory with instrumental texture measurement – an overview of recent developments. Journal of texture, 18 (1) 1–15.

Szczesniak, A.S. (2002) Texture is a sensory property. Food quality and preference 13, 215-225.

Taskaya L., Chen Y.C., Jaczynski J. (2009) Functional properties of proteins recovered from whole gutted silver carp (Hypophthalmichthys molitrix) by isoelectric solubilization/precipitation. LWT – Food Science and Technology. 42, 1082-1089.

Thybo AK., Bechmann IE., Martens M., Engelsen SB. (2000) Prediction of sensory texture of cooked potatoes using uniaxial compression, near-infrared spectroscopy and low-field H-1 NMR spectroscopy. Lebensm.-Wiss u-Technol 33(2) 103-111.

Thyholt K., Isaksson T. (1997) Differentiation of frozen and unfrozen beef using near-infrared spectroscopy. Journal of the Science for Food and Agriculture, 73 (4) 525-532.

Torrisen O.J., Sigurgisladottir S., Slinde E. (2001) Texture and Technological properties of fish. In: Farmed fish quality, Kestin S.C. e Warris P.D. Eds. Oxford UK, 42-57.

Tortonese, E. (1975) *Osteichthyes* (pesci Ossei), Collana Fauna d'Italia, Calderini, Bologna, 2, 636 p.

Tozawa H., Enokihara K., Amano K. (1971) Proposed modification of Dyer's method for trimethylamine determination in cod fish. In Fish inspection and quality control. London: Fishing News Books, 187-190.

Triqui, R. (2006) Sensory and flavor profiles as a means of assessing freshness of hake (*Merluccius merluccius*) during ice storage. Eur Food Res Technol 222, 41–47.

Tsigarida E., Boziaris I., Nychas G. J. (2003) Bacterial synergism or antagonism in a gel cassette system. Applied and Environmental Microbiology 69 (12), 7204–7209.

Usydus Z., Szlinder-Richert J., Adamczyk M. (2009) Protein quality and amino acid profiles of fish products available in Poland. Food Chemistry. 112, 139-145.

Warm K., Boknaes N., Nielsen J. (1998) Development of Quality Index Methods for evaluation of frozen cod (*Gadus morhua*) and cod fillets. J Aquatic Food Prod Technol 7(1), 45-59.

Whittle K., Hardy R., Hobbs G. (1990) Chilled fish and fishery products. In T. Gormley (Ed.), Chilled foods. The state of the art. New York. USA: Elsevier Applied Science, 87-116.

Wold JP., Isaksson T. (1997) Non-destructive determination of fat and moisture in whole Atlantic salmon by near-infrared diffuse spectroscopy. Journal of Food Science, 62 (4), 734-736.

Wong, k., T.A. Gill (1987) Enzymatic determination of trimethylamine and its relationship to fish quality. Journal of Food Science, 52, 1-3.

Yam K.L., Papadakis S.E. (2004) A simple digital imaging method for measuring and analyzing color of food surfaces. Journal of Food Engineering, 61, 137–142.

Zhang HZ., Lee TC. (1997) Rapid near-infrared spectroscopic method for the determination of free fatty acid in fish and its application in fish quality assessment. J Agric. Food Chem. 45 (9), 3515-3521.

Zheng C., Sun D.W., Zheng L. (2006) Recent developments and applications of image features for food quality evaluation and inspection – a review. Trends in Food Science and Technology 17, 642-655.

LIST OF WEBSITES CONSULTED

FAO 2013

http://www.fao.org/fishery/geartype/307/en

http://www.fao.org/wairdocs/tan/x5916e/x5916e01.htm

http://www.fao.org/fishery/geartype/search/en

(19/02/2013)

http://www.fao.org/docrep/007/y5486e/y5486e00.htm

http://www.fao.org/fishery/fishfinder/maps/en

(28/02/2013)

http://www.fao.org/wairdocs/tan/x5916e/x5916e01

(05/03/2013)

FISHBASE, 2013

http://www.fishbase.org/summary/Merluccius-merluccius.html

http://www.fishbase.org/summary/Boops-boops.html

http://www.fishbase.us/summary/Mullus-barbatus+barbatus.html

(07/03/2013)

ISMEA, 2013

http://media.teknoring.it/file/news/Checkup_ittico_2010.pdf

http://www.ismea.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/7868

(07/03/2013)

seafood.ucdavis.edu/pubs/qualitysafety.doc

07/03/2013

Uniprom 2013

http://www.uniprom.it/_cd2/tecniche.htm

28/02/2013

Wikipedia, 2013

http://en.wikipedia.org/wiki/Adriatic_Sea

(5/01/2013)

http://en.wikipedia.org/wiki/Merluccius_merluccius

07/03/2013

Paper I

Red mullet (Mullus barbatus) freshness assessment at two different storage temperatures: comparison among sensorial and physical analyses

Red mullet (*Mullus barbatus*) freshness assessment at two different storage temperatures: comparison among sensorial and physical analyses

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Abstract

The evaluation of fish freshness can be performed using physical, chemical and sensorial methods. Besides sensory methods, several instrumental techniques have been applied with the objective of replacing sensory assessment. The aim of this study was to set-up and test objective physical methods based on computer vision system (CVS), and empirical-rheological texture analysis, to assess red mullet (*Mullus barbatus*) freshness evolution during 10 days of storage at two different storage temperatures (0 and 4 °C). In order to check the effectiveness of the purposed physical methods, CVS features (loss in the pigmentation of the epidermis, development of gill mucus, eye concavity index) and firmness have been compared with sensorial (QIM) attribute scores. Results of this study indicated that the development of physical methods, particularly image analysis performed by CVS, for the evaluation of red mullet freshness is very promising. As expected fish degradation was faster at the higher temperature. Image analysis results demonstrated a high correlation with traditional sensorial freshness assessments. Particularly the eye concavity index showed high positive correlation with sensorial eye ($R^2 > 0.89$) but in particular with the total QIM score ($R^2 > 0.95$).

Keywords

Mullus barbatus, freshness, quality index method, visual quality, computer vision system, texture.

1. Introduction

The quality of fish products is indispensably linked to the freshness of the raw material modulated by appropriate storage conditions, specially the storage temperature after catch (Sikorski et al. 1990).

Characteristic sensorial changes occur in the appearance, odour, taste and texture of fish when deterioration takes place. In Europe, the most commonly used method for the quality assessment of raw fish is the sensorial inspection, and for the fishing industry is the European Union Scheme. This scheme does not take into account differences between species because only general parameters are used. Alternative sensorial methods such as the quality index method (QIM) have been suggested, based on the significant sensory parameters of one specific species (Martinsdóttir et al., 2001).

Post mortem biochemical changes in fish tissues are strongly influenced by the holding temperature after catch, the capture method and the previous condition of the live fish (Sikorski et al. 1990). Therefore post-capture handling of fish affects directly the quality of the product. Primary loss of freshness is caused by endogenous biochemical changes in the muscle, and not by bacterial action, which causes fish to spoil. Freshness declines before the fish is spoiled by microorganisms (Ehira and Uchiyama, 1987).

The evaluation of fish freshness can be performed using physical, chemical and sensorial methods. In some cases it may also be important to assess the evaluation of the associated microbial component with microbiological analysis. Nowadays sensory evaluation is one of the most used methods for the assessment of fish quality and freshness (Martinsdóttir et al., 2001).

The Quality Index Method (QIM) is a seafood freshness quality control system, which shows to be rapid and reliable (Martinsdòttir *et al.*, 2001). QIM is based upon a scheme originally developed by Bremner (1985), and it is based on the observation of characteristic changes that occur overtime in the fish stored in ice. To the changes that affect both the appearance (eyes, skin, gills), and the smell and texture of the sample, it is assigned a score of demerit, specific and free from all the other features. The advantages of QIM Method is the possibility to estimate the passed and the remaining storage time in ice (Botta, 1995).

Besides sensory methods, several instrumental methods have been applied with the objective of replacing sensory assessment.

The assessment of chromatic and geometric features from food image (i.e. colour, size, shape and structure) has been widely applied in the food industry, for quality evaluation and control objectives. Colour characteristics are effective tools for indicating the response of food products, as a consequence of processing and storage. With the remarkable development in

hardware, the use of image data as input features could become realistic, which might be an exceptional indicator of food quality (Zheng et al., 2006). For instance an automated image analysis method was able to detect different visual quality parameters (e.g. cutlet area, dorsal fat depot, red muscle, fat percentage and colour) of rainbow trout cutlets. It was also possible to produce images of cutlets of adequate quality for image analysis, using a simple flatbed scanner (Stien et al., 2006).

Fish may become tough because of *rigor mortis* or frozen storage, or soft and mushy due to autolytic degradation (Huss, 1995). Texture is an important property of fish and its assessment can be performed by empirical-imitative texture analyser instruments (Tryggvadòttir et al., 2001).

Red Mullet (*Mullus Barbatus*) is a member of the *Mullidae* family, living in the Mediterranean and Black seas. It is a benthic species, like the other mullets. This fish is of great commercial value; some papers on proximate and fatty acid composition of this fish is available in the literature (Polat et al., 2009). As the market demand for fresh red mullet is quite high, the study about the freshness decay of this fish is of interest to both retailers and consumers. Ozyurt et al. (2009) studied the shelf-life of red mullet during storage in ice in terms of sensory, microbiological and chemical changes. They found that the sensory acceptability limit was 11 days, with a maximum QIM total demerit score of about 14.

In this direction, the aim of this study was to set-up and test objective physical methods based on computer vision system (CVS), and empirical-rheological texture analysis, to assess fish freshness evolution during storage at two different storage temperatures (0 and 4 °C). In order to check the effectiveness of the purposed physical methods, results have been compared with sensorial (QIM) evaluations.

2. Materials and Methods

2.1 Fish samples

Samples preparation and storage conditions

Fresh red mullet (*Mullus barbatus*) were caught in the Adriatic Sea (Cesenatico, Italy) during Autumn from a fishing vessel. After fishing, the fish were placed in polystyrene boxes, covered with ice flakes and after unloading, sent immediately to the laboratory of the Campus of Food Science. After about 30 minutes, in the laboratory, 80 ungutted fish were individually inserted in open plastic pouches, and placed in polystyrene boxes in two different conditions: 0°C, samples covered with ice flakes (fish-to-ice ratio 2:1), replenishing melted ice daily; 4°C,

samples placed in polystyrene box without ice and in a refrigerate room at 4 °C. Both Boxes were stored in a 4 °C refrigerated room up to 10 days.

2.2 Sensory evaluation

Samples (3 fishes for each sampling time and storage condition) were taken for sensory evaluation at 0, 1, 3, 7 and 10 days of storage. A trained panel of five members evaluated the fish throughout the storage period on each sampling occasion up to 10 days of storage, according to the Quality Index Method (QIM) (Özyurt et al., 2009).

This sensory scale is based on the freshness quality grading system for herring developed by Nielsen and Hyldig (2004).

The QIM involves specifying the characteristics of appropriate sensory attributes of the raw fish, assigning a demerit score ranging from 0 to 1, 2 or 3 depending on the different sensory attribute (**Table 1**). The scores for all characteristics are then summed to give an overall sensory score, the so-called quality index (Botta, 1995). The scale gives zero score for absolutely fresh fish, and increasing total demerit points as fish during deterioration. According to Özyurt et al. (2009), the limit of acceptability for red mullet (*Mullus barbatus*) on this freshness scale was individuated at about 14 of demerits score. The parameters examined were: (1) appearance of skin; (2) blood on gill cover; (3) texture; (4) texture of belly; (5) odour; (6) eyes appearance and shape and (7) gills colour and odour.

1 **Table 1**. QIM scheme for sensory evaluation of red mullet modified by Özyurt et al. (2009)
2

Quality parameter	Description	Score
Whole fish		
Appearance of skin	Very bright	0
	Bright	1
	Mat	2
Blood on gill cover	None	0
	Some	1
	Much	2
Texture	Hard	0
	Firm	1
	Soft	2
Texture of belly	Firm	0
	Soft	1
	Burst	2
Odour	Fresh sea odour	0
	Neutral	1
	Slight off odour	2
	Strong off odour	3
Eyes		
Appearance	Bright	0
	Somewhat lustreless	1

Shape	Convex	0
	Flat	1
	Sunken	2
Gills		
Colour	Characteristic red	0
	Somewhat pale, mat, brown	1
Odour	Fresh, seaweedy, metallic	0
	Neutral	1
	Some off odour	2
	Strong off odour	3

Total demerit points (0-18).

2.3 Texture assessment

For each sampling time, three fish at each temperature condition were taken and after equilibration at 4°C, submitted to texture analysis (one measurement for fish).

Flesh fish firmness was evaluated by performing a penetration test on the dorsal lateral line areas of the fish, using a TA-HDi500 texture analyzer (Stable Micro Systems, Surrey, UK) with a 250 kg load cell. The chose of measuring conditions was carried out after preliminary experiments, where the test was performed on different part of the fish. It was pretty much mandatory to avoid bony plates meet and the thoracic area characterized by nearly every organ. The test was run with a metal probe of 6 mm diameter, and a rate and depth of penetration of 1 mm s^{-1} and 5 mm, respectively. Initially a kind of compression is measured, since the probe does not break the skin and tends to penetrate inside the meat. Reached the maximum limit break, it breaks. The flesh firmness (Fmax, N) was evaluated as the maximum peak detected during penetration, at the point of rupture.

2.4 Visual quality assessment using computer vision system (CVS)

Image acquisition

Visual quality assessment was performed on the same fish for all the periods of analysis (10 fish stored under ice and 10 stored at 4°C). Red mullet images were captured using the image acquisition system developed by Rocculi et al. (2007) with slight modifications. The samples were illuminated using two parallel lamps (with two fluorescent tubes by lamp, model TL-D Deluxe, Natural Daylight, 18W/965, Philips, NY, USA) with a colour temperature of 6500 K (*D*65, standard light source commonly used in food research) and a colour-rendering index (Ra) close to 90%. The four fluorescent tubes (60 cm long) were situated 35 cm above the sample and at an angle of 45° with the sample. Additionally, light diffusers covering each lamp and electronic ballast assured a uniform illumination system. A Color Digital Camera (CDC), Canon PowerShot A70 was located vertically over the sample at a fixed distance. The angle between the camera lens and the lighting source axis was around 45°. Lamps and CDC were inside a wooden box with internal walls that were painted black to avoid the light and reflection from the room. RGB images from the two sides of each fish were taken on the matte black background using the following camera settings: manual mode with the lens aperture at *f* of 4.5 and speed 1/125, no zoom, no flash, 2592 × 1944 pixels resolution of the CDC and storage in JPEG format. The camera was connected to the USB port of a PC provided with a Canon Remote Capture Software (Version 2.7.0) to visualize and acquire the digitalized images directly from the computer.

Image analysis and feature extraction

The CDC was positioned vertically above the sample at a variable distance (d) depending on the particular type of scanning.

The image analysis of sample pictures was performed with an advanced Image Analysis Software (Image-Pro 6.2, Media Cybernetics, USA), using RGB or Gray scale. In this study 3 different parameters was measured: loss in the pigmentation of the epidermis (d=23cm), development of gill mucus and eye concavity index (d=18.5cm).

Loss in the pigmentation of the epidermis

The change in pigmentation of the epidermis (two sides for each fish) was observed on the fish surface. In order to evaluate the modification of the epidermis pigmentation, a chromatic model has been created. Fish lateral images were evaluated in two steps: selection of total sample area (**Figure 1a**), and of area with different pigmentation on it (**Figure 1b**). For this aim, on the

bases of the chromatic characteristics of all samples, a colour model composed of 10 RGB classes was built (**Table 2**). Ten colour classes were created in order to cover 100% of the general surface of fish. The objectification of the classes has been performed on the basis of the operator sensory perception, following a colour change visually detectable from him, thus maintaining a close relationship with the sensory methods used until now. The same colour model was applied to all fish images. The software, examining all pixels in the image, calculates the area percentages of each single class (Figure 1b).

The application of the chromatic model on fish surface images, obtained at different storage time, permitted to monitor the modification of each single class during storage, at the two different temperatures investigated. The percentages of the classes with decreasing trend were added, in order to obtain an average of the epidermis pigmentation loss (EPL, %), considering the initial value of the summed classes equal to 100%.

Development of gill mucus

For the determination of gill mucus (two sides for each fish), gill images were converted in Gray Scale 8BPP. A gray achromatic model (two classes) was created in order to determine the percentage of glossy area, that can be ascribable at the presence of superficial mucus on the gill. This parameter was expressed in terms of area (%) of the considered total area of interest.

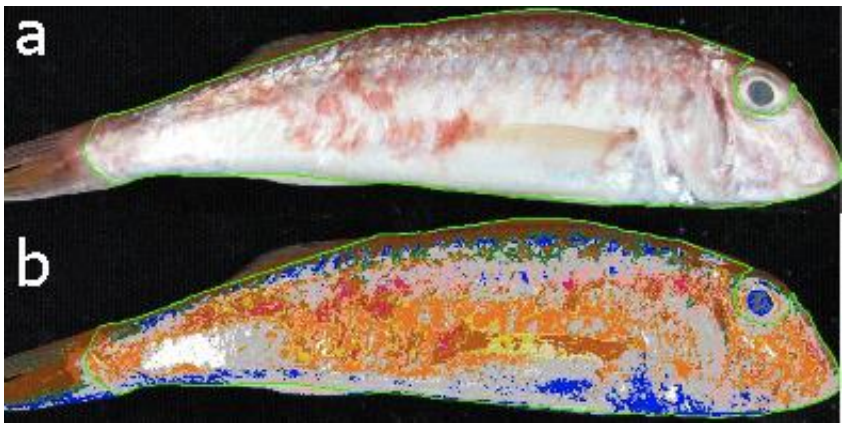

Figure 1. Images of red mullet: a) after the selection of the area of interest; b) after the application of the chromatic model.

Table 2. RGB classes of the chromatic model for the evaluation of the changes of red mullet epiderm pigmentation

Class	Real colour	Colour channel		
		R	**G**	**B**
1	Pink	193-255	164-229	161-239
2	White	239-255	231-255	228-255
3	Gray	161-249	151-252	150-252
4	orange/light brown	216-250	157-216	153-206
5	blue/gray	118-217	201-229	197-224
6	Violaceous	204-216	154-164	204-218
7	light yellow/ light green	227-251	203-244	297-228
8	Brown	128-155	87-127	74-106
9	dark green	149-200	107-116	95-150
10	Pink/very light green	227-252	213-239	217-244

Concavity eye index

One of the most sensitive and therefore perishable part of fish is the eye. It consists of a large proportion of liquid and whose subsequent loss during storage leads to a change in its shape. With the passing of storage time, the eye tends to sink into the eye socket. By measuring the distance that is created as a result of this phenomenon, compared to that of fresh red mullet, the concavity eye index was obtained. In **Figure 2** an image of the red mullet eye of fresh (a) and stored at 4°C for 10 days (b) fish is reported. The concavity eye index (CEI, %) was expressed in terms of percentage increase compared to the initial distance detected (100%).

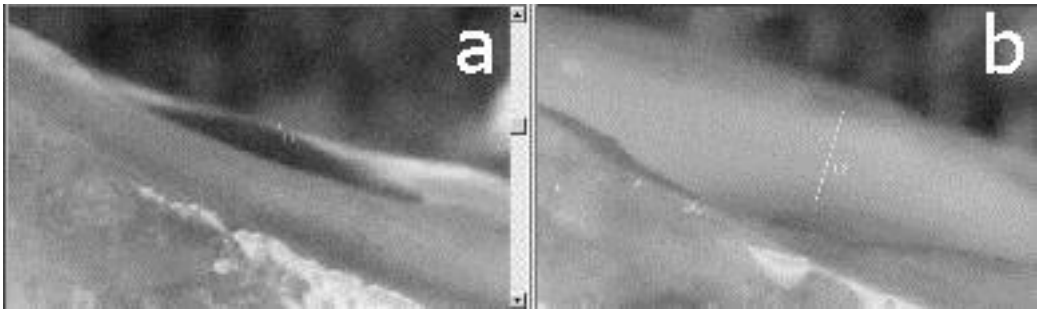

Figure 2. Images of red mullet eye: a) fresh; b) after 10 days of storage at 4°C

3. Results and Discussion

3.1. Sensory assessment

The total demerit points of red mullet stored in ice and at 4°C are reported in **Figure 3**. The initial quality characteristics of the red mullet were a very bright appearance, a hard texture, bright and convex eyes and fresh odours. As expected, demerit points increased at the two different temperatures during storage time. At 4°C the increase of demerit points was faster compared with storage in ice. Özyurt et al. (2009) found a limit of acceptability for red mullet stored in ice, in terms of demerit points, of about 14, and a correspondent storage life of 11 days. According with this finding, in our experimental conditions this limit was reached after about 7 days at 4°C and 10 days at 0°C.

After one day of storage, in terms of firmness (**Figure 4**) both 0 and 4°C samples showed a higher value compared to the fresh sample. Particularly, the sample stored at 0°C reached significantly higher value that refrigerated sample. Considering that this firmness increase was probably due to the incidence of biochemical phenomena bound to *rigor mortis* (Ehira and Uchiyama, 1987), the possible explanation of the detected differences is that at 4°C the occurrence of *rigor mortis* (and of the associated firmness increase) was faster and took place during the first 24 hours of storage (Sikorski et al. 1990).

After *rigor mortis*, both samples showed very similar decreasing value until the end of the experiment, as a consequence of an opposing process to the stiffness occurring in rigor, called tenderization (Careche and Barroso, 2009). This tenderization affects the structural proteins key in the myofibrils and the extracellular matrix, as well as the proteins involved in connections myofibril-myofibril and myofibril-sarcolemma. The extent of these changes and their effect on muscle texture depends on many factors, including species, the pre-slaughter conditions or methods of capture, management and post-mortem treatment and storage time and temperature (Delbarre et al., 2006).

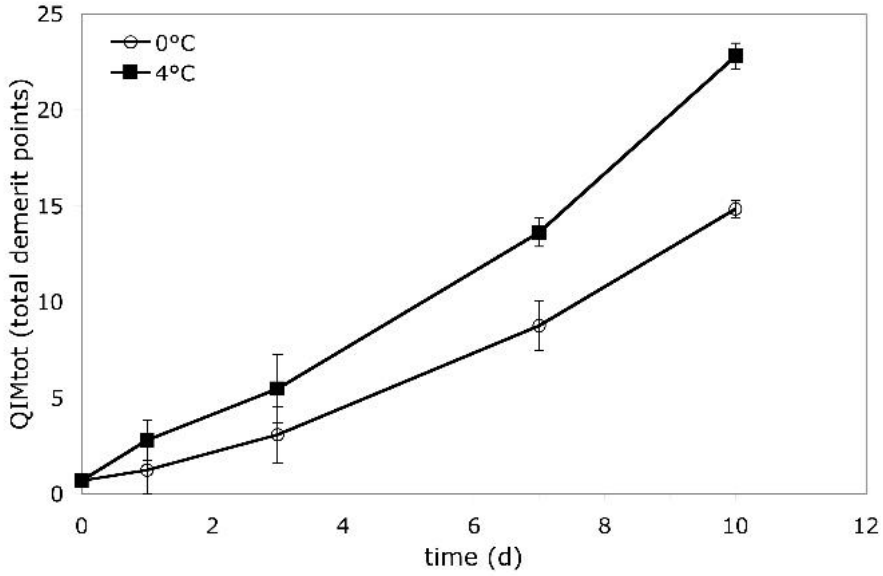

Figure 3. Total demerit points (QIMtot) of red mullet fish stored at 0 and 4°C for 10 days

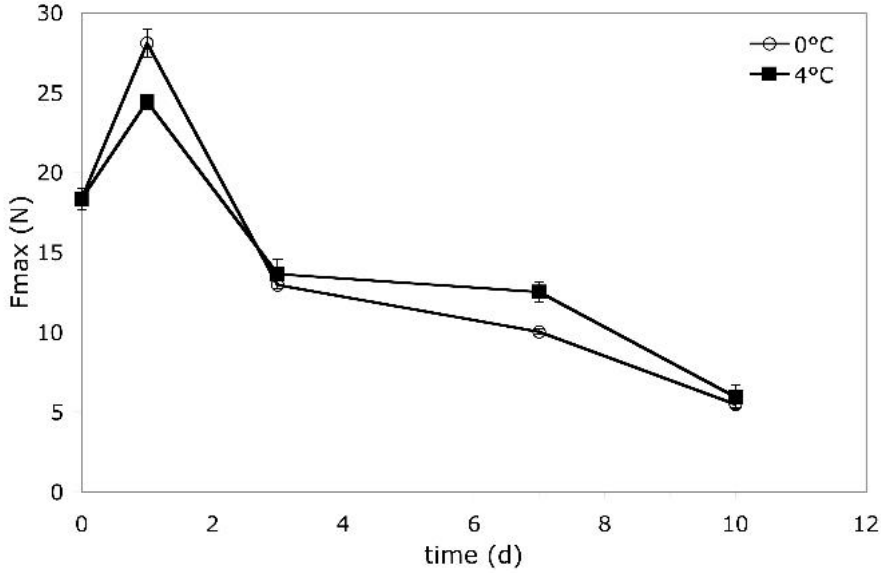

Figure 4. Firmness (Fmax) of red mullet fish stored at 0 and 4°C for 10 days

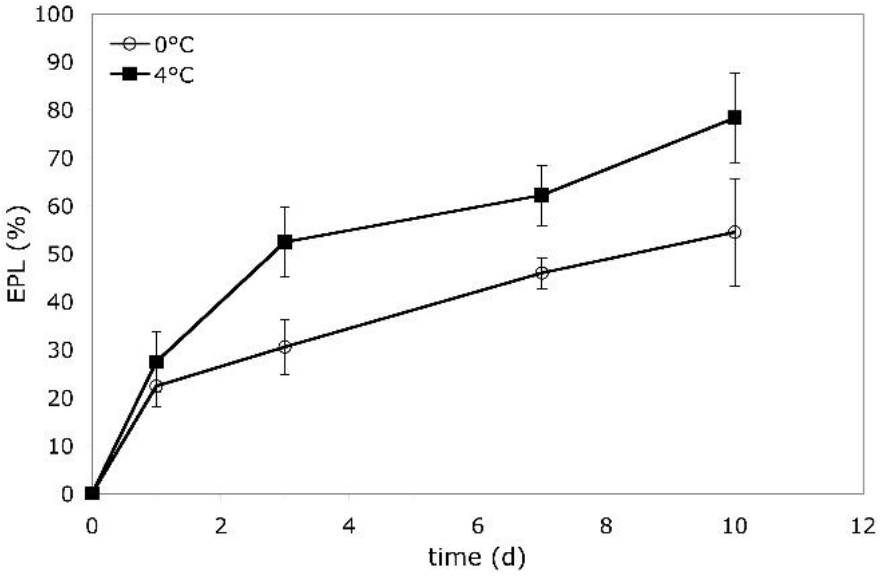

Figure 5. Epidermis pigment loss (%) of red mullet fish stored at 0 and 4°C for 10 days

Results on the evaluation of epidermis pigment modifications during storage are reported in **Figure 5**. During the first day of storage, the degradation of colour for both the samples was very similar, while from the third to the tenth day sample 4°C showed a faster and more intense loss of colour.

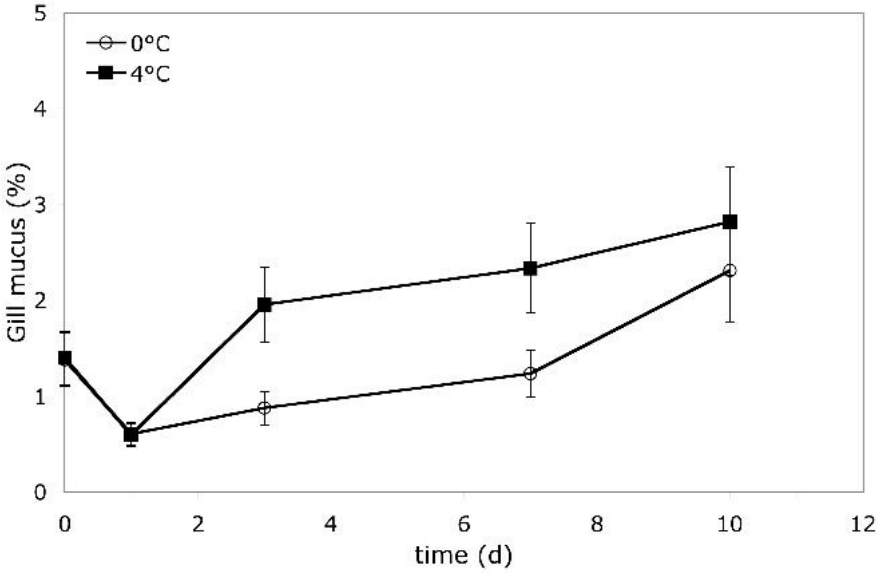

Figure 6. Gill mucus (%) of red mullet fish stored at 0 and 4°C for 10 days

Gill mucus results, in terms of detected glossy area, are reported in **Figure 6**. Both samples showed a slight decrease during the first day of storage, while successively they showed an increasing trend, that was faster and more intense for samples stored at 4°C. Even from a visual examination, the glossy surface of the gills after an initial decrease evidenced a progressive increase. In our opinion, with this CVS technique, it was possible to recognize two different typology of mucus, one peculiar of fresh fish, and the other produced during gill deterioration. A further elaboration of gill images to evaluate also gill distribution could permit the discrimination of the two different kind of mucus.

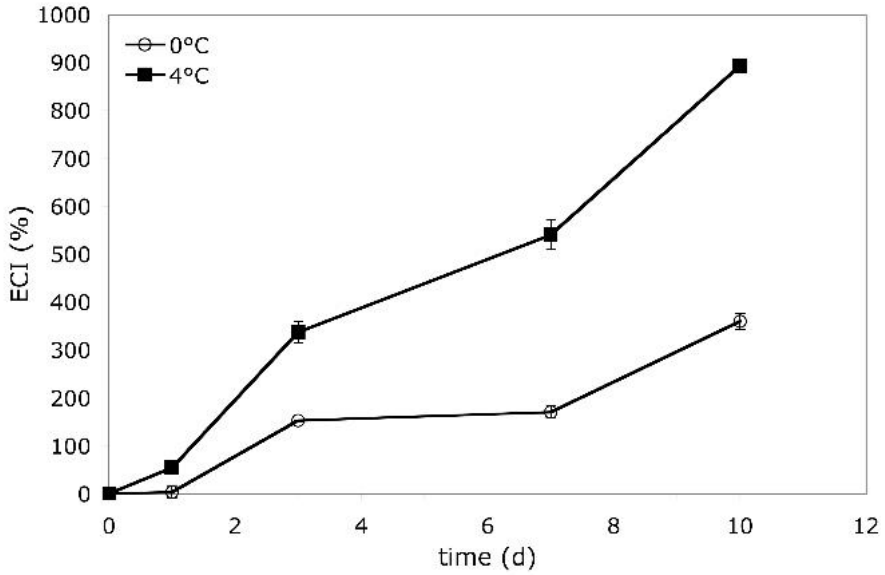

Figure 7. Eye concavity index (%) of red mullet fish stored at 0 and 4°C for 10 days

Data of the eye degradation expressed in terms of eye concavity index (%) are reported in **Figure 7**. Both samples showed an increasing trend, that even for this parameter was higher for sample stored at 4°C, in comparison with those stored under ice. This last condition slightly retarded the beginning of the eye collapse, that was also slower compared with sample stored at 4°C.

4. Conclusions

Results of this study indicated that the development of physical methods, particularly image analysis performed by CVS, for the evaluation of red mullet freshness is very promising. Among CVS parameters, skin colour, presence and distribution of gill mucus, and eye shape

modification evidenced a high sensibility for the estimation of fresh red mullet quality loss, as a function of the two different storage conditions.

As expected fish degradation was faster at the higher temperature; image analysis results demonstrated a high correlation with traditional sensorial freshness assessments. Particularly the eye concavity index detected on fish eye showed high positive correlation with sensorial eye ($R^2 > 0.89$) but in particular with the total QIM score ($R^2 > 0.95$).

5. References

Botta J. F. 1995. Evaluation of seafood freshness quality, New York: VCH Publishers Inc.,180.

Bremner A. 1985. A Convenient, Easy-to-use System for Estimating the Quality of Chilled Seafood. In: D.N. Scott, C. Summers (Eds.*). Proceedings of the Fish Processing Conference, Nelson, New Zealand, 23-25 April 1985.* Fish Processing Bulletin 7, 59-70.

Careche M., Barroso M. 2009. Instrumental texture measurement. In: Fishery Products: Quality, Safety and Authenticity. United Kingdom: Wiley-Blackwell publishers, 214-239.

Delbarre Ladrat C., Cheret R., Taylor R., Verrez-Bagnis V. 2006. Trends in postmortem aging in fish: understanding of proteolysis and disorganization of the myofibrillar structure. Critical Reviews in Food Science and Nutrition, 46, 409-421.

Huss H.H. 1988. Fresh fish-quality and quality changes. Series No. 29, FAO, Danish International Development Agency.

Huss H.H. 1995. *Quality and quality changes in fresh fish.* FAO Fisheries Technical Paper, 348.

Martinsdóttir E., Sveinsdóttir L., Luten J., Schelvis-Smit R., Hyldig G. 2001. Sensory Evaluation of Fish Freshness, QIM Eurofish.

Ehira S., Uchiyama H. 1987. Determination of fish freshness using the k value and comments on some other biochemical changes in relation to freshness. In: D. E. Kramer, J. Liston (Eds.), Seafood quality determination, pp. 185–207. Amsterdam: Elsevier Science Publishers.

Nielsen D., Hyldig G. 2004. Influence of handling procedures and biological factors on the QIM evaluation of whole herring (*Clupea harengus L.*). Food Research International, 37, 975–983.

Özyurt G., Kuley E., Özkütük S., Özogul F. 2009. Sensory, microbiological and chemical assessment of the freshness of red mullet (*Mullus barbatus*) and goldband goatfish (*Upeneus moluccensis*) during storage in ice. Food Chemistry, 114, 505–510

Polat A., Kuzu S., Ozyurt, G., Tokur B. 2009. Fatty acid composition of red mullet (*Mullus barbatus*): A seasonal differentiation. Journal of Muscle Science, 20, 70-78.

Rocculi P., Gómez Galindo F., Mendoza F., Wadsö L., Romani S., Dalla Rosa M., Sjöholm I. 2007. Effects of the application of anti-browning substances on the metabolic activity and sugar composition of fresh-cut potatoes. Postharvest Biology and Technology, 43, 151-157.

Sikorski Z.E., Kolakowska A., Burt J.R. 1990. Postharvest biochemical and microbiological changes. In: Z.E. Sikorski (Editor). Seafood Resources, Nutritional Composition and Preservation, CRC Press, Inc., BocaRaton, FL., pp. 55–75.

Stien L.H., Manne F., Ruohonen K., Kause A., Rungruangsak-Torrissen K., Kiessling A. 2006. Automated image analysis as a tool to quantify the color and composition of rainbow trout (*Oncorhynchus mykiss* W.) cutlets. Aquaculture, 261-705.

Tryggvadóttir S.V., Olafsdóttir G., Einarsson S. 2001. Multisensor for fish: Storage studies of cod. In: Reykjavik and Tromso (Eds). Texture, Electronic Nose, FIGD analysis, RT-Freshmeter and sensory analysis, Project Report 02-01 FAIR CT-98-4076.

Zheng C., Sun D.W., Zheng L. 2006. Recent developments and applications of image features for food quality evaluation and inspection – a review. Trends in Food Science and Technology 17, 642-655.

Paper II

Isothermal calorimetry to predict the stability of fresh fish fillets in modified atmosphere packaging (MAP)

Isothermal calorimetry to predict the stability of fresh fish fillets in modified atmosphere packaging (MAP)

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Modified atmosphere (MAP) combined with refrigerated storage is proved as an efficient preservation method to extend the shelf life of fish and fish products (1). The application of such "soft hurdles" reduces the rate of fish product deterioration and spoilage caused by microbial growth (2).Isothermal Calorimetry studies on microbial metabolism allowed to distinguish microbial heat production into maintenance and growth components (3). Isothermal calorimetry has been used to follow microbial growth in different types of food (e.g. milk, meat and fruit and vegetable products) (4).

In this study we pioneered the use of isothermal calorimetry to evaluate the microbial growth on refrigerated fish fillets under different MA conditions.

Fresh fillets were sealed in glass ampoules and different concentrations of O_2, CO_2 and either N_2 or NO_2 were modulated in the ampoule head space. Four and seven MA conditions were tested respectively on fillets of sardine (*Sardina pilchardus*) and red mullet (*Mullus barbatus*). Heat production was measured for 14 d at 4 °C using a TAM-Air isothermal calorimeter (TA-Instruments, New Castle, USA).

For all O_2 levels tested, measurements on sardine fillets evidenced the occurrence of two distinct peaks. A first peak occurred during the first 24 h of analysis whilst a wider second peak after about one week . In absence of O_2 a small peak probably due to anaerobic fermentation appeared approximately after one week of analysis. In red mullet experiments a significant increase of heat production was detected after about 24 hours of analysis in all tested MA conditions.

By both decreasing O_2 and increasing CO_2 atmosphere concentrations, the main notable effect was a decrease of the recorded thermal power. This may suggest an inhibition of microbial metabolism whose exponential component appeared

both delayed and with a reduced rate. For both the investigated fish species, replacing N_2 with N_2O did not show any additional effect on microbial heat production.

This study proves the suitability of isothermal calorimetry to screen the effect of modified atmosphere conditions on the microbial stability of fish products. Thus isothermal calorimetry monitoring continuously the microbial growth, possibly allows to select the best promising MAP conditions, which will have to be confirmed with plate counts.

References

(1) Sivertsvik M., Jeksrud W. K., Rosnes J. T. 2002. A review of modified atmosphere packaging of fish and fishery products-Significance of microbial growth activity and safety. *Int. J. Food Sci. Technol*. 37, 107-127.

(2) Gram L., Huss H. H. 1996. Microbiological spoilage of fish and fish products. *Int. J. Food Microbiol.* 33, 121-137.

(3) Lars Wadsö and Yujing Li. A test of models for fungal growth based on metabolic heat rate measurements. 2000.

(4) Alklint C, Wadsö L, Sjöholm I (2004) Effects of modified atmosphere on shelf-life of carrot juice. Food Control 15 131–137.

Isothermal calorimetry to predict the stability of fresh fish fillets in modified atmosphere packaging (MAP)

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introduction

- Modified atmosphere (MAP) combined with refrigerated storage is proved as an efficient preservation method to extend the shelf life of fish and fish products (1).
- The application of such "soft hurdles" reduces the rate of fish product deterioration and spoilage caused by microbial growth (2)
- Isothermal Calorimetry studies on microbial metabolism allowed to distinguish microbial heat production into maintenance and growth components (3).
- Isothermal calorimetry has been used to follow microbial growth in different types of food (e.g. milk, meat and fruit and vegetable products) (4).

aim

In this study we pioneered the use of isothermal calorimetry to evaluate the microbial growth on refrigerated fish fillets under different MA conditions.

materials and methods

- Fresh fillets were sealed in glass ampoules and different concentrations of O_2, CO_2 and either N_2 or NO_2 were modulated in the ampoule head space.
- Three and seven MA conditions were tested respectively on fillets of sardine (*Sardina pilchardus*) and red mullet (*Mullus barbatus*).
- Heat production was measured for 20 days at 10° C using a TAM-Air isothermal calorimeter (TA-Instruments, New Castle, USA).

results and discussion

For all O_2 levels tested, thermal power measurements on sardine fillets (Fig. 1) evidenced the occurrence of two distinct peaks. A first peak appeared during the first 48 h of analysis whilst a wider second peak after about one week.
By imposing '100% O_2' (Fig.1, blue curve) was observed a delay in the occurrence of the second peak, which also showed a broader area than the peaks recorded in 'Air' or in '21%O_2 + 79% N_2O' (Fig.1, green and red curves).

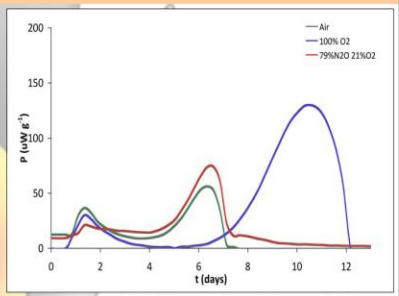

Figure 1. Thermal power measurements on sardine fillets in Air (green curve), 21%O2+79%N2O (red curve) and 100% O2 (blue curve).

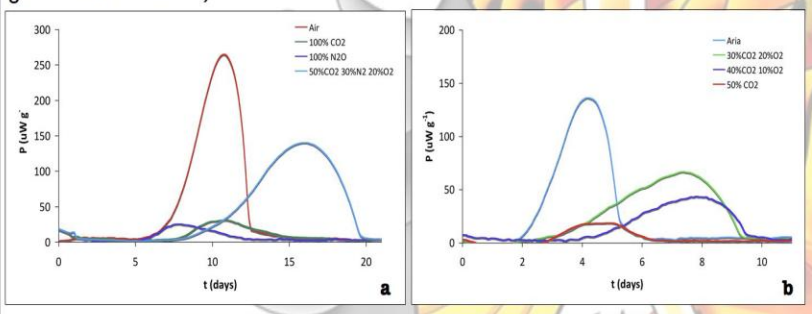

Figure 2. Thermal power measurements on red mullet fillets in Air (a, blue light curve; b, red curve), 30%CO_2+20%O_2 (a, green curve), 40%CO_2+10%O_2 (a, blue curve), 50%CO_2 (a, red curve), 100% CO_2 (b, green curve), 100% N_2O (b, blue curve), 50%CO_2+30%N_2+20%O_2 (b, blue light curve).

In red mullet experiments a significant increase of heat production was detected in all tested MA conditions. However the onset of microbial growth appeared after about 2 and 6 days of analysis (respectively in Fig. 2a and Fig. 2b) as a possible consequence of the different contamination level of the raw material (wild red mullet).
By both decreasing O_2 and increasing CO_2 atmosphere concentrations, the main notable effect was a decrease of the recorded thermal power. This may suggest an inhibition of microbial metabolism whose exponential component appeared both delayed and with a reduced rate.

For both the investigated fish species, replacing N2 with N2O did not show any additional effect on microbial heat production.

conclusions

This study proves the suitability of isothermal calorimetry to screen the effect of modified atmosphere conditions on the microbial stability of fish products. Thus isothermal calorimetry monitoring continuously the microbial growth, possibly allows to select the best promising MAP conditions, which will have to be validated on the real product.

references

(1) Sivertsvik M., Jeksrud W. K., Rosnes J. T. 2002. A review of modified atmosphere packaging of fish and fishery products-Significance of microbial growth activity and safety. Int. J. Food Sci. Technol. 37, 107-127.
(2) Gram L., Huss H. H. 1996. Microbiological spoilage of fish and fish products. Int. J. Food Microbiol. 33, 121-137.
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Paper III

A foodomics approach to evaluate the freshness of selected fish species in different aquaculture systems

A foodomics approach to evaluate the freshness of selected fish species in different aquaculture systems

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The definition of quality related to seafood is really complex. From an objective point of view, it can be defined as "a combination of such characteristics as wholesomeness, integrity and freshness"; otherwise, from the consumers point of view, the quality is strictly connected to the nutritional value, flavor and others sensory components. For all kind of seafood, freshness is essential for the quality of the final product and conventionally is expressed by the Quality Index (QI) mainly based on the sensorial characteristics. Besides QI, there are many chemical methods used for estimating the freshness of fish. Generally the most common ones evaluate the loss of freshness caused by endogenous biochemical changes in muscle: measuring products or by-products of protein breakdown, the degradation of trimethylamine oxide (TMAO), and biochemical indices of quality based on nucleotide degradation (hypoxanthine and "K" value). By using a "Foodomics" approach, based on the NMR spectroscopy, it is possible to evaluate all these parameters in one shot fast analysis. Additionally, it is also possible to obtain holistic descriptors, not depending on the a priori system knowledge, as a function of the spectroscopic molecular profile of the aqueous extracts. Such descriptors are based on the first principal components (1) calculated for different species, e.g. Gilthead sea bream (*Sparus Aurata*) and European seabass (*Dicentrarchus labrax*). In order to evaluate the effectiveness of a calculated metabolic score based on NMR profiles to describe the overall freshness, an experimental work has been conducted on fish specimens stored for 15 days at two different temperatures (4°C and under melting ice) (2). The results show that, even with only 4°C of difference, the kinetics of the molecular profile evolution during time storage, is substantially different. Interestingly, the signals that represent and characterize the kinetics of evolution do not belong to the metabolites that conventionally are used to describe the freshness of fish, but also to other compounds whose meaning will be described in relationship to specific farming conditions (3).

Acknowledgements:
This work was supported by the Italian Ministry of Agricultural, Food and Forestry Policies (Italy) (MiPAAF "FreshFish" Project). API (Associazione Piscicoltori Italiani) is gratefully acknowledged for providing fish samples.

References:
1. Picone, G., Mezzetti, B., Babini, E., Capocasa, F., Placucci, G., Capozzi, F. (2011) Unsupervised Principal Component Analysis of NMR Metabolic Profiles for the Assessment of Substantial Equivalence of Transgenic Grapes (Vitis vinifera) *J. Agric. Food Chem*. 59, pp. 9271-9279.
2. Picone, G., Engelsen, S. B., Savorani, F., Testi, S., Badiani, A., Capozzi, F. (2011) Metabolomics as a Powerful Tool for Molecular Quality Assessment of the Fish Sparus aurata, Nutrients 3, pp. 212-227.
3. Savorani, F., Picone G., Badiani, A., Fagioli, P., Capozzi, F., Engelsen, S. B. (2010) Metabolic profiling and aquaculture differentiation of gilthead sea bream by ^{1}H NMR metabonomics. Food Chem. 120, pp. 907-914.

11th International Conference on the Application of Magnetic Resonance in Food

A FOODOMICS APPROACH TO EVALUATE THE FRESHNESS OF SELECTED FISH SPECIES IN DIFFERENT AQUACULTURE SYSTEMS

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INTRODUCTION

The definition of quality related to seafood is really complex. From an objective point of view it can be defined as "a combination of such characteristics as wholesomeness, integrity and freshness" [1]. There are many methods used for the estimation of the "degree of freshness" in fish. Generally the most common ones evaluate the loss of freshness caused by endogenous biochemical changes in muscle: measuring products or by-products of protein breakdown, the degradation of trimethylamine oxide (TMAO), or lipid oxidation and biochemical indices of quality based on nucleotide degradation (hypoxanthine and "K" value). By using a "Foodomics" approach, based on the NMR spectroscopy, it is possible to evaluate all these parameters with one shot, additionally it is also possible to obtain an holistic descriptor not based on the a priori system knowledge, which is a function of the spectroscopic molecular profile of the aqueous extracts easily prepared.

METHODS

Samples

Gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) (Fig. 1) samples were harvested from two different aquaculture systems: tanks and cages. Ungutted fishes were divided into two groups. The first was stored at 4 °C and samples taken 0, 1, 3, 4, 5, 6, 7, 8, 9, 10, 11 days after catching. The second group was stored on ice and sampled 0, 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 15 days after catching. The fishes were prepared for further processing by slicing them in a room at 4 °C and storing the flesh at -80°C. At each sampling time and temperature a trichloroacetic acid extraction (TCA) was performed on three fish samples, by following the procedure set up by Boland *et* al [2].

^{1}H-NMR Measurements

The samples for NMR analysis were prepared according to Ciampa *et* al. [3]. ^{1}H-NMR spectra were recorded at 298 K with a Bruker (Milan, Italy) AVANCE spectrometer operating at a frequency of 600.13 MHz. Each spectrum was acquired using 32 K data points over a 7211.54 Hz spectral width and adding 256 transients. A recycle delay of 5 s and a 90° pulse of 11.4 μs were set up. Saturation of residual water signal was achieved by irradiating it during the recycle delay at δ equal to 4.703 ppm. Each spectrum was processed with Top Spin 3.0 and applying a line broadening factor of 0.5 Hz [3].

Chemometric Tools

PCA and PLS tools have been applied for extracting relevant information from the acquired NMR data.

RESULTS AND DISCUSSION

1) A typical ^{1}H-NMR spectrum acquired on aqueous fish extracts is shown in Figure 2. Three groups of peaks could be identified, namely i) organic acids and amino acids, between 0.5 and 2.5 ppm; ii) trimethylamine-N-oxide (TMAO), trimethylamine (TMA), creatine and phosphocreatine, accounting for 30% of the total spectra area, corresponding to the signals with the highest intensity between 2.5 and 4.0; iii) the peaks pertaining to the molecules employed to calculate the K-index, in the region between 8.1 and 8.6 ppm. As the ^{1}H-NMR spectrum can give an overview on whole the metabolites present in the sample with one shot, is possible then to evaluate the effect of rearing conditions, temperature and time of storage, considering the whole molecular profile at the same time. However, the complexity of information laying in the spectrum needs to be condensed in order to find out a unique parameter able to describe the loss quality and the evolution of freshness, during time of storage as a function of external factors. The multivariate analysis of NMR data allows to have a score able to summarizes all of the hundreds of parameters, giving thus a measure of the molecular quality of fish

$$\text{Index 1} = \alpha^*par_1 + \beta^*par_2 + \gamma^*par_3 + \ldots\ldots + \omega^*par_{22000}$$

where

Index 1 = K-index, TMA, histamine, fatty acids and aminoacid

2) A PCA analysis (Fig. 3) of data set at T0, after alignment, normalization and mean-centering of spectra, was performed. For both rearing conditions in "sea cages" and "tanks", each species of fish describes two molecular trajectory along the principal feature (PC1), related to both freshness loss and to rearing conditions. On the other side, the second components (PC2) is related to the kind of rearing and to fish' species.

3) The effect of storage time on the molecular profiles of fishes reared in different conditions can also be caught by the same PCA when including samples stored both for 11 days at 4°C and 15 days under ice (Fig. 4).

4) At the end, it is possible to use the NMR freshness index for providing the days of storage at 4°C as shown in figure 5, obtained by applying a PLS-DA [4] on both wild and reared samples.

REFERENCES

[1] Martin, R. (1988). Contaminants in relation to the quality of seafood. Food Technol. 42, pp. 104.

[2] Boland, F.E. Trimethylammine nitrogen in seafood method colorimetric (1971). J. Assoc. Off. Anal. Chem., 54, 9.

[3] Ciampa, A., Picone, G., Laghi, L., Nikzad, H., Capozzi, F. (2012). Changes in the Amino Acid Composition of Bogue (*Boops boops*) Fish during Storage at Different Temperatures by ^{1}H-NMR Spectroscopy. Nutrients 2012, 4, 542-553.

[4] Savorani, F., Picone, G., Badiani , A., Fagioli, P., Capozzi, F., Engelsen, S.B. (2010). Metabolic profiling and aquaculture differentiation of gilthead sea bream by ^{1}H NMR metabonomics. Food Chemistry 120, 907-914.

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FIGURES

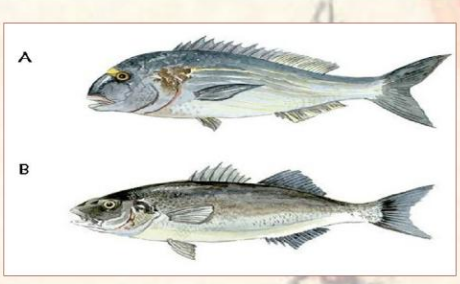

Figure 1. Representation of A) Gilthead sea bream (*Sparus aurata*) and B) European sea bass (*Dicentrarchus labrax*), most important marine fish species

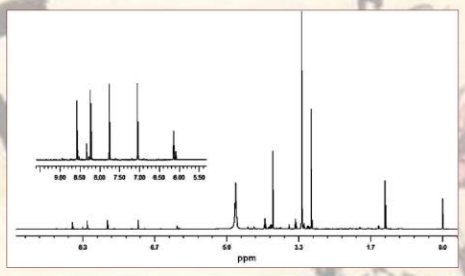

Figure 2. 600.13 MHz ^{1}H NMR spectrum of TCA fish extract. Metabolites assignment is reported by Ciampa *et* al. [3]

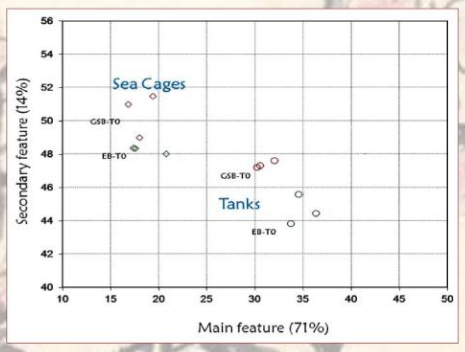

Figure 3. PCA scores plot of samples of Gilthead sea bream (*Sparus Aurata*) in red and European sea bass (*Dicentrarchus labrax*) in green stored at two temperatures.
GSB: Gilthead sea bream; **EB:** European sea bass

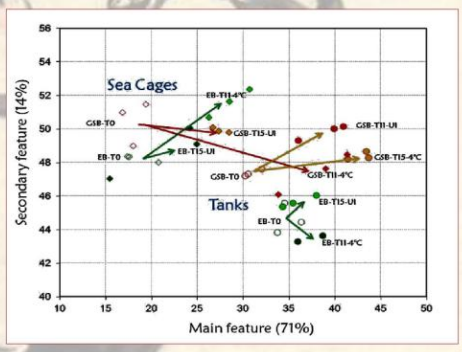

Figure 4. Evolution of freshness during storage, from T0 to T11 (4°C) and T15 (UI).
After 11 days some samples overlapped as it can be seen for Gilthead sea bream. This makes difficult to distinguish samples according to rearing conditions

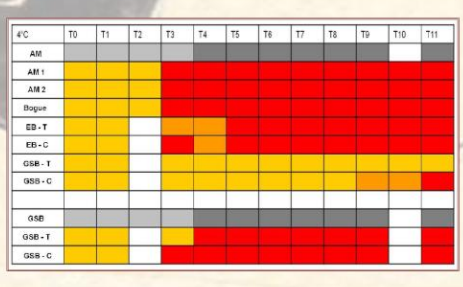

Figure 5. PLS-DA result. In yellow, fresh samples correctly predicted. In red samples misclassified and in orange ambiguous samples. The model ha been built using only samples at 4°C.
AM: Atlantic mackerel (*Scomber scombrus*)

Paper IV

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Article

Changes in the Amino Acid Composition of Bogue (*Boops boops*) Fish during Storage at Different Temperatures by ^{1}H-NMR Spectroscopy

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Abstract: Nuclear magnetic resonance spectroscopy was employed to obtain information about the changes occurring in Bogue (*Boops boops*) fish during storage. For this purpose, ^{1}H-NMR spectra were recorded at 600 MHz on trichloroacetic acid extracts of fish flesh stored over a 15 days period both at 4 °C and on ice. Such spectra allowed the identification and quantification of amino acids, together with the main organic acids and alcohols. The concentration of acidic and basic free amino acids was generally found to increase and decrease during storage, respectively. These concentration changes were slow during the first days, as a consequence of protein autolysis, and at higher rates afterward, resulting from microbial development. Two of the amino acids that showed the greatest concentration change were alanine and glycine, known to have a key role in determining the individual taste of different fish species. The concentration of serine decreased during storage, as highlighted in the literature for frozen fish samples. Differences in the amino acids concentration trends were found to be related to the different storage temperatures from day 4 onwards.

Keywords: fish; freshness index; ^{1}H-NMR; free amino acids (FAAs); quality assessment; metabolic profile; storage

1. Introduction

The amino acid composition of fish muscle proteins has been known for a long time to be remarkably constant across different species of fish [1]. Fish muscles contain from 1 to 5 g of free amino acids for every 100 g of protein, characterized by high quantities of taurine, histidine, glutamic acid, alanine, aspartic acid, leucine and lysine and by lower quantities of cysteine, tryptophan, methionine and tyrosine [2]. The pattern of free amino acid concentrations, rather than the composition of the amino acids bound to the proteins, is known to depend on the fish species. One of the reasons is that some free amino acids act as cell osmoregulators, so that their relative amount with respect to other free amino acids that are not involved in cellular osmotic regulation, can be modulated by the salt concentration in the fish habitat [1].

Concentration and pattern of free amino acids are also very sensitive to the changes occurring in fish muscle during storage. During the first hours following death such characteristics are modulated by autolysis, the degradation of muscle constituents by endogenous enzymes. This degradation is known to be pH, temperature and fish processing dependent [3].

The pH, decreasing upon death due to the transformation of glycogen into lactate, modulates the exit of the lytic enzymes from lysosomes. In this respect, it is of importance to note that catching methods that involve intense fish struggling lead to a greater transformation of glycogen into lactic acid, thus to a more powerful autolysis. The rates of these reactions are generally directly related to temperature, with an optimum around 36 °C for most of the lytic enzymes. Among the processing treatments able to modulate autolysis, evisceration may be of primary importance, as some proteolytic enzymes are known to be located in the gut [4].

The greatest modifications to the amount and pattern of free amino acids occur when the conditions are favorable for bacterial development. This phenomenon has contrasting effects on free amino acid concentrations as these molecules are extracted from proteins by lytic reactions and, in parallel, transformed into secondary products, some of which lead to malodors [5].

Autolysis and bacterial spoilage are responsible for changes occurring in the concentrations of adenosine-5′-triphosphate (ATP), adenosine-5′-diphosphate (ADP), adenosine-5′-monophosphate (AMP), and inosine-5′-monophosphate (IMP), which are quantitatively converted to inosine (HxR) and hypoxanthine (Hx) [6,7]. For this reason, all such molecules are often quantified to evaluate the quality loss during fish storage, and their relative amounts are combined together in a score to express fish freshness. One of the first conventional quality indices setup to take the degradation of such nucleotides into consideration is the K index [8], defined as

$$K\ (\%) = ([HxR] + [Hx])/([ATP] + [ADP] + [AMP] + [IMP] + [HxR] + [Hx])$$

As free amino acids are key molecules in both autolysis and biological spoilage reactions, their observation may offer alternatives to the K index and its variants to follow fish quality loss during

storage. Proton nuclear magnetic resonance (^{1}H-NMR) can be in turn a suitable technique for this purpose, as suggested by recent papers published by some of the authors of the present work [9].

Picone *et al*. [10] observed free amino acids and other low molecular weight molecules using ^{1}H-NMR to discriminate farmed specimens of Gilthead Seabream (*Sparus aurata*) according to the aquaculture system employed. Savorani *et al*. [11] employed NMR spectra to find out discriminant NMR intervals' spectra through *i*ECVA [11,12], whilst Capozzi *et al*. [13] outlined a new algorithm that was able to correct the dilution errors during sample preparation affecting the quantification of serine, valine, histidine, phenylalanine and other amino acids.

Prompted by these encouraging results, we decided to apply ^{1}H-NMR spectroscopy to monitor the changes occurring in the free amino acid pool during fish storage, to investigate the possibility of using such changes as storage quality indices. The fish chosen for the investigation belong to the Bogue (*Boops boops*) species, whose amino acidic composition is still uncharacterized in the literature, even if this species is commercially important and very popular in several Mediterranean countries [14,15].

2. Experimental Section

2.1. Sampling

All samples of Bogue fish, provided by Magna Grecia Mare—Portus Veneris (Leuca, Lecce, Italy), were caught in spring and brought to the laboratory in about ten hours, by means of polystyrene boxes filled with ice flakes. Ungutted fishes were divided into two groups. The first was stored at 4 °C and samples taken 0, 2, 4, 6, 8, 10, 11 days after catching. The second group was stored on ice and sampled 0, 2, 4, 6, 8, 10, 15 days after catching. The fishes were prepared for further processing by slicing them in a room at 4 °C and storing the flesh at −80 °C.

2.2. Sample Preparation for ^{1}H-NMR Analysis

At each sampling time and temperature a trichloroacetic acid extraction (TCA) was performed on three fish samples, by following the procedure set up by Boland *et al* [16]. For this purpose, 25 g of fish muscle was added to 50 mL of 7.5% (w/w) TCA and minced by means of a vertical homogenizer (Ultra-Turrax, Ika®). The resulting product was filtered with filter paper (No. 4) from Whatman (Little Chalfont, Buckinghamshire, HP7 9NA, UK). The pH of a 1 mL aliquot was adjusted to 7.8 using 9 M KOH in an Eppendorf microfuge tube and centrifuged at 14 K rpm for 5 min in order to remove potassium trichloroacetate precipitate. The so obtained supernatant was stored at −80 °C until ^{1}H-NMR measurements were performed.

2.3. ^{1}H-NMR Measurements

The samples were prepared for NMR analysis by adding 160 μL of a D_2O solution of 3-(trimethylsilyl)-propionic-2,2,3,3-d4 acid sodium salt (TSP) 6.25 mM to the thawed samples.

^{1}H-NMR spectra were recorded at 298 K with a Bruker (Milano, Italy) AVANCE spectrometer operating at a frequency of 600.13 MHz, equipped with an autosampler with 60 holders.

Each spectrum was acquired using 32 K data points over a 7211.54 Hz spectral width and adding 256 transients. A recycle delay of 5 s and a 90° pulse of 11.4 μs were set up. Acquisition time (2.27 s)

and recycle delay were adjusted to be 5 times longer than the T_1 of the protons under investigation, which has been considered to be not longer than 1.4 s. Saturation of residual water signal was achieved by irradiating it during the recycle delay at δ equal to 4.703 ppm. Each spectrum was processed with MestReC 4.9.8.0 (Mestreab Research SL, Spain) by manually adjusting phase and base-line and applying a line broadening factor of 0.5 Hz.

The peaks were assigned by comparing their chemical shift and multiplicity with the literature [11]. When peaks due to different protons of the same molecule were identified, both were employed for the quantification.

3. Results and Discussion

A typical ^{1}H-NMR spectrum obtained during the present investigation is shown in Figure 1. Three groups of peaks could be identified. The peaks with the highest intensity, accounting for 30% of the total spectra area, pertain to trimethylamine-*N*-oxide (TMAO), trimethylamine (TMA) and creatine and phosphocreatine. In the region between 8.16 and 8.60 ppm the peaks employed to calculate the K index could be identified. The remaining peaks could be mainly ascribed to amino acids and, to a minor extent, to organic acids and short chain fatty acids. At the chemical shifts of the amino acids sharp peaks could be identified, with a width at half height around 1 Hz, which could be assigned to amino acids in the free form or pertaining to low molecular weight peptides. At the base of some of such peaks, macromolecules and aggregates gave rise to much broader signals, which could not be discriminated from the baseline noise. Such different behavior is caused by the inverse relationship existing between signal linewidth and (i) the nuclear relaxation rate, being shorter for slow tumbling macromolecules; and (ii) molecular anisotropy, being longer for denatured and disordered proteins [17].

Figure 1. 600.13 MHz ^{1}H NMR spectrum of TCA Bogue fish extract. The numbers refer to the assignments reported in Table 1.

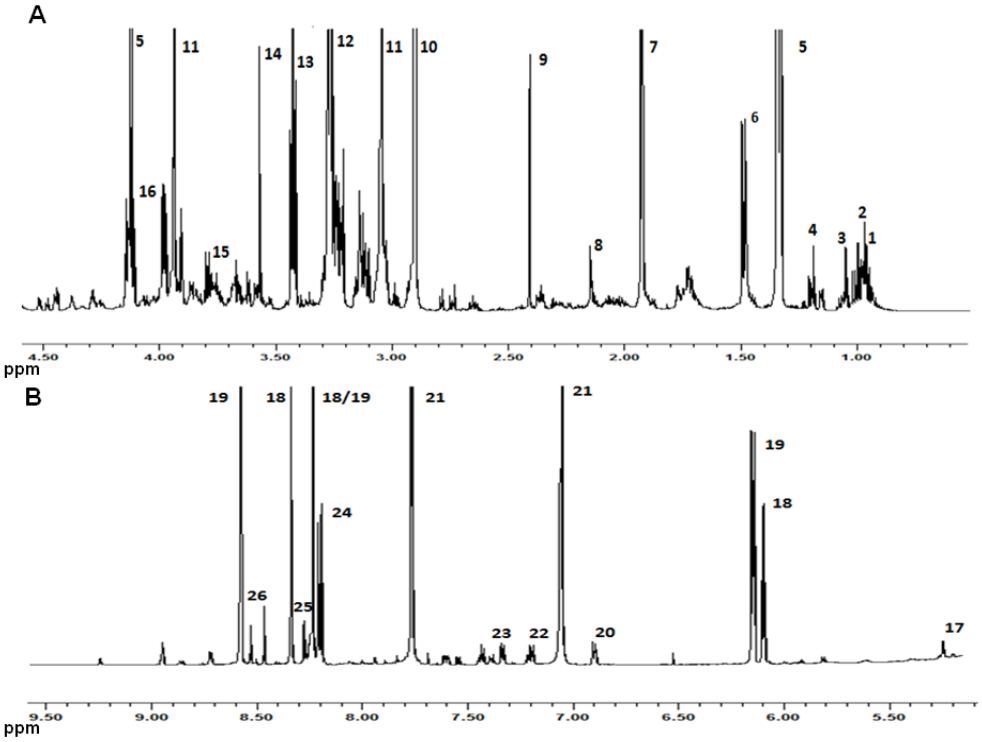


Table 1 lists the NMR signals of the amino acids involved in the fish spoilage during the present investigation. The resonances used in the integration were chosen from among those belonging to the corresponding signal multiplet which was found to be only marginally overlapping with other signals. Nevertheless, for some amino acids a clean signal was not found, and for this reason their variation was given in a cumulative way (e.g., Ile, Leu and Val) or not given at all (e.g., Lys and Pro). In addition, the table lists the assignments of other substances, whose concentration could help in understanding which phenomenon, autolysis or bacteria development, was mainly responsible for the identified fluctuations of the amino acid concentrations.

Table 1. Summary of the metabolites identified in the 600.13 MHz ^{1}H NMR spectrum of the aqueous extract of Bogue fish.

Compound	Assignment	^{1}H (ppm)	Multiplicity
Isoleucine (Ile) [1]	δ-CH_3	0.94	t
Leucine (Leu) [2]	δ′-CH_3	0.96	d
Valine (Val) [3]	γ-CH_3-γ′-CH_3	1.00–1.05	dd
Ethanol [4]	CH_3	1.19	t
Lactate (La) [5]	β-CH_3	1.33	d
Alanine (Ala) [6]	β-CH_3	1.49	d
Acetate [7]	CH_3	1.93	s
Methionine (Met) [8]	S–CH_3	2.14	s
Succinate [9]	α,β-CH_2	2.41	s
Trimethylamine (*N*-TMA) [10]	N–CH_3	2.90	s
Creatine/Phosphocreatine [11]	N–CH_3 and N=C	3.04	s
Oxide Trimethylamine (*N*-TMAO) [12]	N–CH_3	3.27	S
Taurin (Tau) [13]	N–CH_2	3.42	t
Glycine (Gly) [14]	α-CH	3.56	s
Glutamate (Glu) [15]	α-CH	3.75	t
Creatine/Phosphocreatine [11]	N–CH_2	3.94	s
Serine (Ser) [16]	β-CH	3.98	dd
α-Glucose (α-GLC) [17]	CH-1	5.24	d
Inosine (HxR) [18]	CH-1′, ribose	6.10	d
Inosine 5′-monophosphate (IMP) [19]	CH-1′, ribose	6.14	d
Tyrosine (Tyr) [20]	$C_{3,5}$H, ring	6.88	d
Histidine (His) [21]	C_2H ring/C_4H ring	7.06/7.77	s
Tryptophan (Trp) [22]	C_5H ring	7.19	t
Phenylalanine (Phe) [23]	CH-2,6	7.32	m
Hypoxanthine (Hx) [24]	CH-8	8.19	s
Hypoxanthine (Hx) [24]	CH-2	8.21	s
Inosine (HxR) [18]	CH-8	8.233	s
Inosine 5′-monophosphate (IMP) [19]	CH-8	8.236	s
Adenosine 5′-triphosphate (ATP) [25]			
Adenosine 5′-diphosphate (ADP) [25]	CH-8	8.27	s
Adenosine 5′-monophosphate (AMP) [25]			
Inosine (HxR) [18]	CH_2, ring	8.33	s
Formate (Fo) [26]	CH	8.46	s
Inosine 5′-monophosphate (IMP) [19]	CH_2, ring	8.57	s

When the content evolution of a mixture has to be evaluated by comparing different spectra registered from different samples, the spectra cannot be directly compared, even when acquired with the same parameters as in the present case. Several factors are in fact known to alter the sensitivity of the instrument from sample to sample, leading to variations in the relationship between concentration of the analytes and the area of the corresponding peaks [18].

To avoid this source of error when measuring content evolution of a mixture, most often a referring standard is selected whose concentration is constant among the different samples. The spectra intensity is then scaled to this concentration, a procedure called "normalization" or vertical scaling [13].

When dealing with biological samples, it is common practice to employ the total area of the spectrum as a referring standard, assuming that all the substances interconvert to each other during storage, leading to an almost constant total area [19]. This option could not be utilized for the samples analyzed during the present investigation, as protein hydrolysis occurring during conservation led to a progressive increase of the spectral total area. The same phenomenon potentially influences the concentration of every molecule characterizing the samples, so that none of them could be confidently employed as an endogenous internal standard. Another possibility with biological samples consists in adjusting the spectral vertical scale to the area of an added molecule, the so-called added internal reference standard. When the investigated samples are liquids extracted from solids, the standard addition can only be in the last step of the sample preparation process. The relative area of the internal standard becomes thus sensitive to the variability induced by the extraction efficiency, which in turn is variable among samples undergoing time dependent structural degradation [20]. In addition, an added molecule may represent an unreliable concentration reference if interactions with macromolecules characterizing the sample occur [21].

An alternative to all the above mentioned normalization procedures can be offered by the presence of a small pool of metabolites known to be present in similar concentrations in every sample and known to belong to a closed inter-conversion pathway, so that their total molar amount can be considered as constant during storage. This is the case of the ATP degradation pathway, which in *post mortem* conditions is ultimately converted to hypoxanthine, via the by-products included in the calculation of the K-index [6]. To test the possibility of scaling towards the area of the peaks due to ATP and byproducts, a procedure we may call K-index normalization (KIN), the total area of these molecules was calculated on the raw spectra before normalization. In spite of the possible sources of error potentially affecting the spectra, the samples analyzed at the same storage times gave an average RSD of 3.3% and no statistically significant differences were found among the time points. The KIN method was thus elected as the normalization procedure for the present work.

Figures 2 and 3 present the fluctuation during storage on ice and at 4 °C of the molecules followed during the present investigation. The amino acids could be divided into three groups according to the trend of their concentration. The first group was represented by taurine only, whose concentration did not significantly change during storage. This was not unexpected, since this amino acid is not employed by organisms as a protein constituent, thus it is not involved in the lytic processes going on during autolysis reactions or bacterial development. Indeed, results in accordance with this observation can be found in the literature for several kinds of fishes and fish preparations, stored from −20 °C [22] to 25 °C [23].

Figure 2. Concentration changes relative to fresh samples of taurine, histidine and serine during storage at 4 °C (blue symbols and lines) and on ice (red symbols and lines).

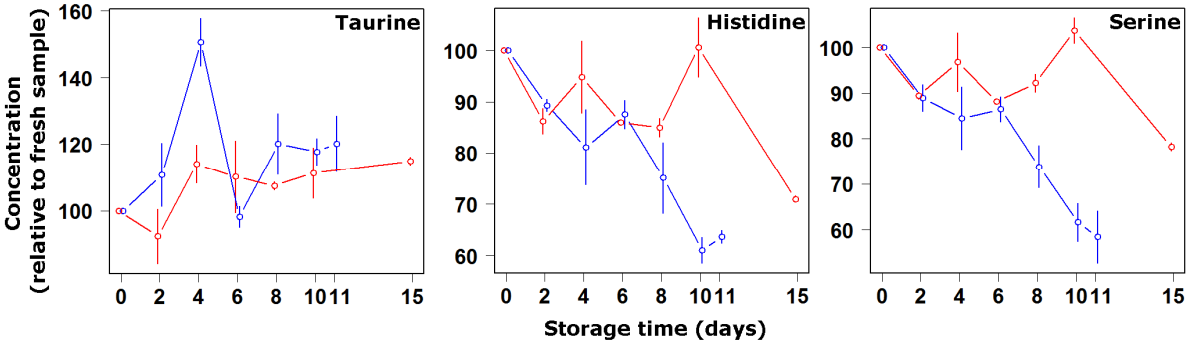
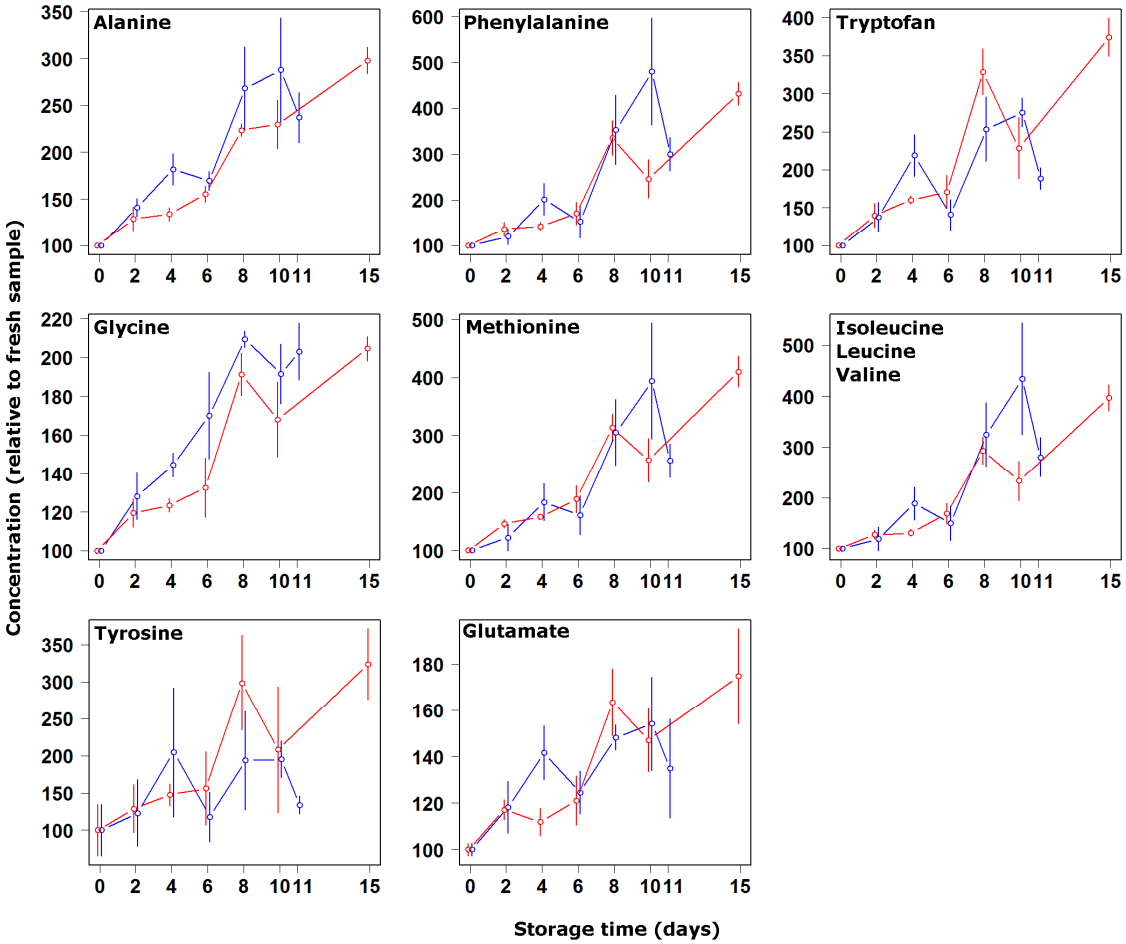


Figure 3. Concentration changes relative to fresh samples of alanine, phenylalanine, tryptophan, glycine, methionine, isoleucine-leucine-valine, tyrosine and glutamate during storage at 4 °C (blue symbols and lines) and on ice (red symbols and lines).



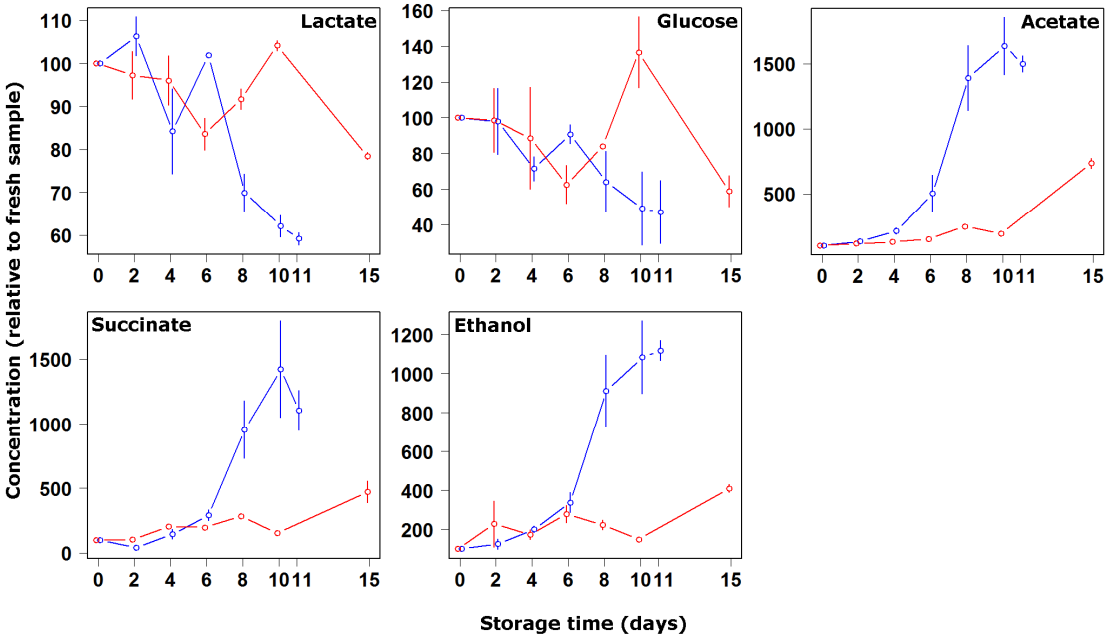
The second and third groups comprised the amino acids whose concentration increased and decreased, respectively, as a consequence of storage at 4 °C. The former group was composed of alanine, phenylalanine, tryptophan, glycine, methionine, isoleucine-leucine-valine, tyrosine and glutamate, while the latter group was made up of histidine and serine only. The decrease in concentration of the basic amino acids shows that their solubilization from muscle proteins was slower

than their transformation into byproducts, namely biogenic amines through decarboxylation. Such finding, together with the parallel increase of the acidic amino acids, is well documented at ambient temperature for a variety of fishes and transformed products based on them [24,25]. Kiesvaara [26] set up specific quality indices for salted herrings based on such knowledge. At room temperature, moreover, there is a general agreement about the decrease of methionine during storage [27]. The data collected for the present investigation seems to confirm the findings. Exceptions are represented by serine, which is acidic but decreases, and methionine, which increased in concentration at both investigated temperatures. Such trends appear to be more similar to those observed in the literature for frozen samples. As an example, Jiang *et al*. [22] found decreasing concentrations of serine in frozen samples of mackerel, amberfish, mullet and carp during storage, and at the same time an increasing concentration of methionine. The fact that at both temperatures the tested methionine did not decrease is interesting from a consumer perspective, as the catabolism of this amino acid is known to be primarily responsible for methylmercaptan and dimethylsulphide formation, molecules strongly related to development of off-flavors [28].

By focusing on the relationship between amino acid concentrations and storage time, it was possible to divide the observed molecules into two categories. The concentration of alanine, tryptophan, glycine methionine and tyrosine were observed to change during storage with a constant trend. In contrast, the other molecules seemed to be characterized by a slow change until day 4 and by a higher change rate afterwards. In this respect it must be noted that the concentration of some of the latter molecules, in particular histidine and serine, changed at similar rates in the two storage methods until day 4, and at markedly different rates afterwards. Such a two-phase observation could be rationalized considering that the enzymes leading to autolysis are known to be poorly influenced by temperature, being still active even at temperatures as low as −17 °C [3]. The slow rate change until day 4 can thus be considered mainly due to autolysis, the fast rate change occurring afterwards due to bacterial development. Indeed, the concentration of some of the mentioned molecules seem to reproduce a bacterial development curve [5] characterized by: (i) a lag phase in which the Bogue can be considered as fresh. During this phase the concentration of some amino acids undergoes fluctuations appreciated by the consumer in specific cases. Free glycine, for example, is known to be important for the individual taste of different fish species [29]; (ii) an exponential multiplication phase, typically characterized by development of off-flavors; and (iii) a stationary phase during which the concentration of some free amino acids start to decrease.

To confirm the findings based on the quantification of the free amino acids, other molecules involved in the microorganisms' development were quantified (Figure 4). Glucose and lactate are recognized as a substrate for most of the microorganisms involved in the spoilage of fish, glucose being consumed at first instance, followed by lactate and amino acids [30]. At the same time, acetate, succinate and ethanol typically accumulate in the medium, as a consequence of such development. Indeed, during the present investigation a 40% decrease of both glucose and lactate was noticed for the samples storage at 4 °C, whilst acetate, succinate and ethanol increased from the fourth day in samples stored at 4 °C and after 15 days in the samples stored on ice.

Figure 4. Concentration changes relative to fresh samples of lactate, glucose, acetate, succinate and ethanol during storage at 4 °C (blue symbols and lines) and on ice (red symbols and lines).



4. Conclusions

In the present work, changes of Bogue fish muscle composition were followed as a consequence of storage at 4 °C and on ice. For this purpose, ^{1}H-NMR spectra were recorded on Bogue fish muscle TCA extracts and then normalized to the total area of the peaks pertaining to ATP and its degradation products. Through such amplitude adjustment, preferred to other kinds of vertical scaling procedures described in the literature, the effects of storage on the concentration of 13 amino acids in the free form could be registered. The concentration of some of them was observed to increase in the fish flesh as a consequence of enzymatic reactions, during the first days of storage, and due to bacterial development afterwards. Histidine concentration was observed to decrease, as a consequence of decarboxylation leading to histamine. The storage temperature seemed to mainly affect bacterial development rate, modulating the amino acid concentrations starting from day 4 of storage. The amino acids profile was shown to be sensitive to the phenomena leading to the compositional changes occurring during fish storage. Its evaluation, through ^{1}H-NMR spectroscopy or other less expensive techniques, appears to be a promising source of parameters suitable for the assessment of freshness, as an alternative to the K-index or analogue indices. It is important to stress here that NMR spectroscopy must be considered as a "non targeted" technique able to quantify and evaluate kinetics for unselected compounds, even *a posteriori*, e.g., after a pool of spectra is recorded on a population of samples and the multivariate analysis points out some interesting features in the molecular profile, that may be suitable for the development of new parameters related to quality and freshness.

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Conflict of Interest

The authors declare no conflicts of interest relating to this manuscript.

References

1. Bramstedt, F. Amino acid composition of fresh fish and influence of storage and processing. In *Fish in Nutrition*; Heen, E., Kreuzer, R., Eds.; Fishing News (Books) Ltd.: London, UK, 1962; pp. 61–67.
2. Connell, J.J.; Howgate, P.F. The amino-acid composition of some british food fishes. *J. Sci. Food Agric.* **1959**, *10*, 241–244.
3. Mukundan, M.K.; Antony, P.D.; Nair, M.R. A review on autolysis in fish. *Fish Res.* **1986**, *4*, 259–269.
4. Voskresensky, N.A. *Fish as Food*; Academic Press: New York, NY, USA, 1965; Volume 3, pp. 107–131.
5. Gram, L.; Huss, H.H. Microbiological spoilage of fish and fish products. *Int. J. Food Microbiol.* **1996**, *33*, 121–137.
6. Mendes, R.; Quinta, R.; Nunes, M.L. Changes in baseline levels of nucleotides during ice storage of fish and crustaceans from the portuguese coast. *Eur. Food Res. Technol.* **2001**, *212*, 141–146.
7. Márquez-Ríos, E.; Morán-Palacio, E.F.; Lugo-Sánchez, M.E.; Ocano-Higuera, V.M.; Pacheco-Aguilar, R. Postmortem biochemical behavior of giant squid (*Dosidicus gigas*) mantle muscle stored in ice and its relation with quality parameters. *J. Food Sci.* **2007**, *72*, C356–C362.
8. Saito, T.; Arai, K.; Matsuyoshi, M. A new method for estimating the freshness of fish. *Bull. Jpn. Soc. Sci. Fish.* **1959**, *24*, 749–750.
9. Capozzi, F.; Cremonini, M.A. Nuclear magnetic resonance spectroscopy in food analysis. In *Handbook of Food Analysis Instruments*; Otles, S., Ed.; CRC Press: Boca Roton, FL, USA, 2008; pp. 281–318.
10. Picone, G.; Balling Engelsen, S.; Savorani, F.; Testi, S.; Badiani, A.; Capozzi, F. Metabolomics as a powerful tool for molecular quality assessment of the fish *Sparus aurata*. *Nutrients* **2011**, *3*, 212–227.
11. Savorani, F.; Picone, G.; Badiani, A.; Fagioli, P.; Capozzi, F.; Engelsen, S.B. Metabolic profiling and aquaculture differentiation of gilthead sea bream by ^{1}H-NMR metabonomics. *Food Chem.* **2010**, *120*, 907–914.
12. Nørgaard, L.; Bro, R.; Westad, F.; Engelsen, S.B. A modification of canonical variates analysis to handle highly collinear multivariate data. *J. Chemom.* **2006**, *20*, 425–435.

13. Capozzi, F.; Ciampa, A.; Picone, G.; Placucci, G.; Savorani, F. Normalization is a Necessary Step in NMR Data Processing: Finding the Right Scale Factors. In *Magnetic Resonance in Food Science: An Exciting Future*; Renou, J.P., Belton, P.S., Webb, G.A., Eds.; CRC Press: Cambridge, UK, 2011; pp. 147–160.
14. Özogul, Y.; Özogul, F. Fatty acid profiles of commercially important fish species from the mediterranean, aegean and black seas. *Food Chem.* **2007**, *100*, 1634–1638.
15. Koutsoumanis, K.; Nychas, G.-J.E. Chemical and sensory changes associated with microbial flora of mediterranean boque (Boops boops) stored aerobically at 0, 3, 7, and 10 °C. *Appl. Environ. Microbiol.* **1999**, *65*, 698–706.
16. Boland, F.E. Trimethylammine nitrogen in seadfood method colorimetric. *J. Assoc. Off. Anal. Chem.* **1971**, *54*, 9.
17. Bordoni, A.; Picone, G.; Babini, E.; Vignali, M.; Danesi, F.; Valli, V.; Di Nunzio, M.; Laghi, L.; Capozzi, F. NMR comparison of *in vitro* digestion of *Parmigiano reggiano* cheese aged 15 and 30 months. *Magn. Reson. Chem.* **2011**, *49*, S61–S70.
18. Burton, I.W.; Quilliam, M.A.; Walter, J.A. Quantitative ^{1}H-NMR with external standards: Use in preparation of calibration solutions for algal toxins and other natural products. *Anal. Chem.* **2005**, *77*, 3123–3131.
19. Ndagijimana, M.; Laghi, L.; Vitali, B.; Placucci, G.; Brigidi, P.; Guerzoni, M.E. Effect of a synbiotic food consumption on human gut metabolic profiles evaluated by 1 h nuclear magnetic resonance spectroscopy. *Int. J. Food Microbiol.* **2009**, *134*, 147–153.
20. Craig, A.; Cloarec, O.; Holmes, E.; Nicholson, J.K.; Lindon, J.C. Scaling and normalization effects in NMR spectroscopic metabonomic data sets. *Anal. Chem.* **2006**, *78*, 2262–2267.
21. Kriat, M.; Confort-Gouny, S.; Vion-Dury, J.; Sciaky, M.; Viout, P.; Cozzone, P.J. Quantitation of metabolites in human blood serum by proton magnetic resonance spectroscopy. A comparative study of the use of formate and tsp as concentration standards. *NMR Biomed.* **1992**, *5*, 179–184.
22. Jiang, S.T.; Lee, T.C. Changes in free amino acids and protein denaturation of fish muscle during frozen storage. *J. Agric. Food Chem.* **1985**, *33*, 839–844.
23. Je, J.-Y.; Park, P.-J.; Jung, W.-K.; Kim, S.-K. Amino acid changes in fermented oyster (*Crassostrea gigas*) sauce with different fermentation periods. *Food Chem.* **2005**, *91*, 15–18.
24. Ababouch, L.; Afilal, M.E.; Benabdeljelil, H.; Busta, F.F. Quantitative changes in bacteria, amino acids and biogenic amines in sardine (*Sardina pilchardus*) stored at ambient temperature (25–28 °C) and in ice. *Int. J. Food Sci. Technol.* **1991**, *26*, 297–306.
25. Özden, Ö. Changes in amino acid and fatty acid composition during shelf-life of marinated fish. *J. Sci. Food Agric.* **2005**, *85*, 2015–2020.
26. Kiesvaara, M. *On the Soluble Nitrogen Fraction of Barrel-Salted Herring and Semi-Preserves During Ripening*; Valtion Teknillinen Tutkimuskeskus: Espoo, Finland, 1975.
27. Love, R.M. *The Chemical Biology of Fishes: Advances 1968–1977, with a Supplementary Key to the Chemical Literature*; Academic Press: San Diego, CA, USA, 1980.
28. Herbert, R.A.; Shewan, J.M. Precursors of the volatile sulphides in spoiling north sea cod (*Gadus morhua*). *J. Sci. Food Agric.* **1975**, *26*, 1195–1202.

29. Giannakourou, M.C.; Koutsoumanis, K.; Nychas, G.J.E.; Taoukis, P.S. Development and assessment of an intelligent shelf life decision system for quality optimization of the food chill chain. *J. Food Prot.* **2001**, *64*, 1051–1057.
30. Dainty, R.H. Chemical/biochemical detection of spoilage. *Int. J. Food Microbiol.* **1996**, *33*, 19–33.

Paper V

Microbiological effects of on-board fishing vessel handling in Merluccius merluccius

EFFETTI MICROBIOLOGICI DELLA MANIPOLAZIONE A BORDO DEL PESCHERECCIO IN MERLUCCIUS MERLUCCIUS

MICROBIOLOGICAL EFFECTS OF ON-BOARD FISHING VESSEL HANDLING IN MERLUCCIUS MERLUCCIUS

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SUMMARY

The purpose of the present study was to determine the impact of different manipulation techniques applied on board fishing vessel, on the microbiological quality of the flesh of European hake (*Merluccius merluccius*) during storage at +3°C ± 1°C for a time (T) of 10 days after landing (T1-T10). Samples of fish were taken from a fishing vessel of the Adriatic Sea and from one of the Tyrrhenian Sea, treated on-board under different icing conditions: 1) a low ice/product weight ratio and 2) an optimal ice/product weight ratio, up to 1:3 (3). Spoilage bacteria as Total Bacterial Count (TBC) and specific spoilage bacteria as Sulphide Producing Bacteria (SPB) were enumerated in fish flesh as Colony Forming Units (CFU/g) on Plate Count Agar and Lyngby Agar at 20°C for 3-5 days. TBC of the Adriatic fishes (gutted on-board) resulted 10^3 UFC/g at T1-T6, and 10^4-10^5 at T10, whereas TBC of the Tyrrhenian fishes (not gutted on-board) resulted 10-10^2 UFC/g at T2-T3, 10^3 at T6, and 10^4-10^5 at T10. SPB resulted 10- 10^2 UFC/g at T1-T6, and 10^3-10^4 at T10, with absolute values higher in the Adriatic fishes, in respect with the Tyrrhenian fishes, and in the low icing conditions in respect with the optimal icing condition. At the experimented condition, the lowering of the microbiological quality of fish flesh during storage, seems to be more dependent on the gutting versus not gutting on-board practice rather than on the low versus optimal icing treatment.

KEYWORDS European hake, icing conditions on-board, TBC, SPB

INTRODUZIONE

È ampiamente riconosciuto che la qualità dei prodotti ittici dipende strettamente dalla corretta manipolazione e dal mantenimento della catena del freddo. Nei pesci sani e subito dopo la morte, le carni sono sterili, e la flora microbica contaminante è localizzata esclusivamente sulla superficie esterna, sulle branchie e nel tubo digerente.

I fenomeni autolitici, che si realizzano precocemente (1), sono determinati da numerosi enzimi, tra cui predominano fosforilasi, lipasi, catepsine ed altri enzimi intestinali (2) e comportano modificazioni della consistenza e della permeabilità dei tessuti, favorendo la migrazione dei batteri verso le carni. In particolare si ritiene che i batteri presenti a livello branchiale raggiungano le carni attraverso il sistema vascolare, mentre i batteri intestinali oltrepassano attivamente la parete e la membrana, e quelli presenti sulla superficie corporea attraversano la cute (3,4). La colonizzazione delle masse muscolari dei pesci, nelle quali la proliferazione batterica è favorita dal collasso del sistema immunitario (5), è dunque un fenomeno inevitabile, che comunque può essere condizionato dalle modalità di manipolazione e conservazione comprese quelle effettuate a bordo dei pescherecci. La quantità

della ghiacciatura in particolare, è considerata indispensabile per il mantenimento delle caratteristiche organolettiche, quali la rigidità post-mortem e la compattezza delle carni, e per il contenimento dei processi di degradazione derivanti dall'attività enzimatica endogena e dalla proliferazione batterica.
Ovviamente il rapporto in peso ghiaccio/prodotto varia a seconda della temperatura ambientale, ma escludendo le aree tropicali, un rapporto 1/3 può essere considerato ottimale (6). Studi precedenti condotti sulla sardina, mantenuta integra a temperatura di refrigerazione, mostrano che al terzo giorno di conservazione è apprezzabile un discreto aumento della componente batterica nei filetti (7), e condizioni analoghe sono state riscontrate nell'acciuga (dati non pubblicati).
Per quanto riguarda la pratica della eviscerazione, secondo il Reg. CE 853/2004, essa andrebbe praticata "al più presto possibile", anche se numerosi autori riportano di non aver rilevato differenze significative di contaminazione microbica nei filetti di pesci di diverse specie sottoposti e non alla asportazione del pacchetto intestinale e successiva conservazione in ghiaccio (8), mentre secondo studi condotti sullo scorfano del pacifico, si evidenziano condizioni ottimali di conservazione proprio nei soggetti non eviscerati (9).
In questa sede vengono presentati i risultati delle valutazioni di carattere microbiologico effettuate su filetti di nasello (*Merluccius merluccius*) conservato a temperatura di refrigerazione, ottenuti nell'ambito di uno studio multidisciplinare finanziato dal Ministero delle Politiche Agricole ed ancora in corso, mirato alla verifica dell'impatto sulla qualità del pescato della applicazione di diverse modalità di manipolazione a bordo dei pescherecci.

MATERIALI E METODI

L'indagine è stata svolta su diversi lotti di nasello provenienti dall'Adriatico e dal Tirreno, confezionati a bordo del peschereccio in cassette di polistirolo secondo due modalità: tesi 1 con ghiacciatura considerata scarsa e tesi 2 con ghiacciatura considerata ottimale (fondo e lati della cassetta, più idonea copertura del prodotto), secondo un rapporto ghiaccio/prodotto di circa 1:3.
I campioni dell'Adriatico sono stati eviscerati a bordo, e consegnati al laboratorio lo stesso giorno del landing, mentre quelli del Tirreno, non eviscerati, sono stati mantenuti a temperatura di refrigerazione e consegnati il giorno successivo.
I controlli sono stati effettuati a partire dal giorno dopo la consegna mantenendo il lotto campionario a +3°C ± 1°C fino a 10 giorni.
Le analisi microbiologiche sono state effettuate sui filetti relativamente a Conta Batterica Totale (CBT), quale indice generico di contaminazione e Batteri Produttori di Solfuri (BPS), quale indice specifico di spoilage, mediante la conta in piastra delle Unità Formanti Colonia (UFC/g) a 20°C per 3-5 gg, utilizzando il Plate Count Agar (PCA) (Oxoid) ed il Lyngby Agar, terreno di elezione per la quantificazione sia della CBT che dei BPS (10).
Per la valutazione statistica dei dati è stato applicato il test di significatività t-student, utilizzando Microsoft Excel 2003, relativamente alle tre variabili CBT su PCA, CBT su LYA e BPS su LYA, ed alle due tesi a confronto, T1 (ghiacciatura scarsa), T2 (ghiacciatura ottimale).

RISULTATI

Si riportano i dati relativi a 7 cicli di osservazione, di cui 4 con prodotto del Tirreno e 3 con prodotto dell'Adriatico, per un totale di 168 soggetti esaminati. I risultati ottenuti vengono presentati in forma grafica (fig. 1-6).

Nel prodotto proveniente dall'Adriatico i valori di CBT rilevati su PCA (fig. 1) e LYA (fig. 2) sono sostanzialmente sovrapponibili e dell'ordine di 3 log dal T1 al T3 in entrambe le tesi. Al T6 i valori mantengono lo stesso logaritmo, ma risultano leggermente superiori nella tesi con ghiacciatura scarsa su entrambi i terreni. Al T10 i valori aumentano fino a 5 log su entrambi i terreni, tranne la tesi con ghiacciatura scarsa che non supera i 4 log su LYA. I valori relativi ai BPS (fig. 3) al T1 risultano pari ad 1 log in entrambe le tesi.
Dal T1 al T6 i valori della tesi con ghiacciatura scarsa aumentano progressivamente fino ai 2 log, mentre risultano sostanzialmente stabili quelli della tesi con ghiacciatura ottimale. Al T10 i valori di entrambe le tesi raggiungono i 5 log.
Nel prodotto proveniente dal Tirreno i valori di CBT rilevati su PCA (fig. 4) e LYA (fig. 5) al T2 sono pari ad 1 log nella tesi con ghiacciatura ottimale, e circa 2 log nella tesi con ghiacciatura scarsa. Al T3 entrambe le tesi mostrano valori superiori ma sempre dell'ordine di 2 log con leggera prevalenza nella tesi con ghiacciatura scarsa e su LYA. Al T6 entrambe le tesi mostrano valori di 3 log, leggermente superiori nella tesi con ghiacciatura scarsa.
Al T10 i valori della tesi con ghiacciatura ottimale raggiungono i 4 log e quelli della tesi

con ghiacciatura scarsa i 5 log, su entrambi i terreni. I valori relativi ai BPS (fig. 6) risultano pari a 1 log, con tendenza all'aumento dal T2 al T3 in entrambe le tesi. Al T6 i valori raggiungono i 2 log, ed al T10 i 3 log, in entrambe le tesi.

Figura 1. Conta Batterica Totale (CBT) su PCA a 20°C, in nasello dell'Adriatico (eviscerato), conservato a +3°C ± 1°C. Manipolazione a bordo: Tesi 1 ghiacciatura scarsa (-G), Tesi 2 ghiacciatura ottimale (+G). Tempo espresso in giorni (T1-T10).

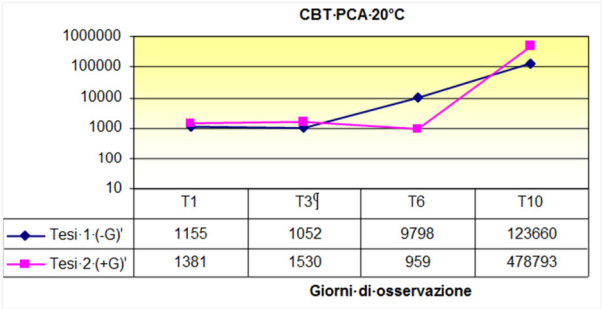


Figura 2. Conta Batterica Totale (CBT) su LYA a 20°C, in nasello dell'Adriatico (eviscerato), conservato a +3°C ± 1°C. Manipolazione a bordo: Tesi 1 ghiacciatura scarsa (-G), Tesi 2 ghiacciatura ottimale (+G). Tempo espresso in giorni (T1-T10).

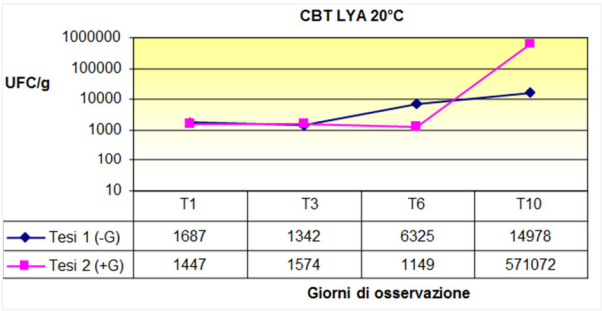


Figura 3. Conta Batteri produttori di H_2S (BPS) su LYA a 20°C, in nasello dell'Adriatico (eviscerato), conservato a +3°C ± 1°C. Manipolazione a bordo: Tesi 1 ghiacciatura scarsa (-G), Tesi 2 ghiacciatura ottimale (+G). Tempo espresso in giorni (T1-T10).

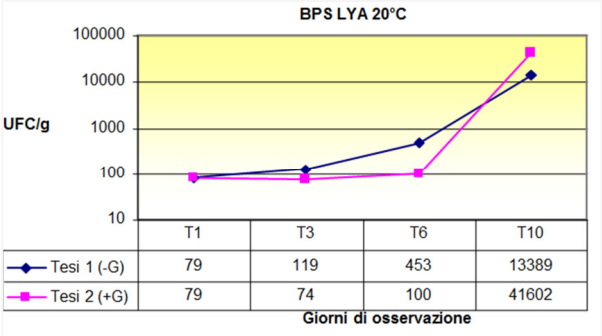


Figura 4. Conta Batterica Totale (CBT) su PCA a 20°C, in nasello del Tirreno (non eviscerato), conservato a +3°C ± 1°C. Manipolazione a bordo: Tesi 1 ghiacciatura scarsa (-G), Tesi 2 ghiacciatura ottimale (+G). Tempo espresso in giorni (T1-T10).

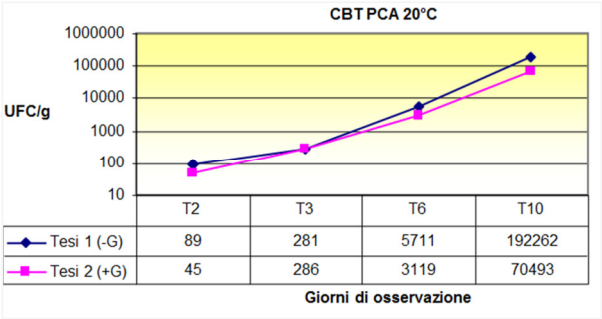


Figura 5. Conta Batterica Totale (CBT) su LYA a 20°C, in nasello del Tirreno (non eviscerato), conservato a +3°C ± 1°C. Manipolazione a bordo: Tesi 1 ghiacciatura scarsa (-G), Tesi 2 ghiacciatura ottimale (+G). Tempo espresso in giorni (T1-T10)

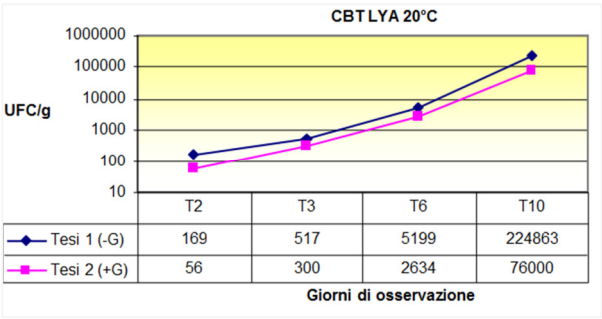
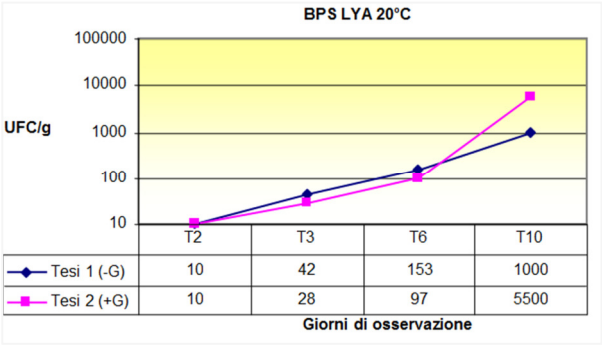


Figura 6. Conta Batteri produttori di H_2S (BPS) a 20°C, in nasello del Tirreno (non eviscerato), conservato a +3°C ± 1°C. Manipolazione a bordo: Tesi 1 ghiacciatura scarsa (-G), Tesi 2 ghiacciatura ottimale (+G). Tempo espresso in giorni (T1-T10).



I risultati della elaborazione statistica dei dati per il confronto fra le 2 tesi sono riportati nelle tabelle 1-6. Per nessun tempo di osservazione la differenza tra le medie geometriche è risultata significativamente diversa.

Tabella 1. Analisi statistica relativa alla CBT su PCA a 20°C in naselli dell'Adriatico. Tesi 1 (ghiacciatura scarsa) versus tesi 2 (ghiacciatura ottimale).

Giorni di conservazione	T1		T3		T6		T10	
Rapporto tra le medie (=diff)	0,836		0,649		10,074		0,258	
IC95% (min;max)	0,012	58,522	0,012	33,935	0,030	3330,319	0,001	77,932
p-value	0,912		0,777		0,294		0,546	

Tabella 2. Analisi statistica relativa alla CBT su LYA a 20°C in naselli dell'Adriatico. Tesi 1 (ghiacciatura scarsa) versus tesi 2 (ghiacciatura ottimale).

Giorni di conservazione	T1		T3		T6		T10	
Rapporto tra le medie (=diff)	1,166		0,663		4,929		0,026	
IC95% (min;max)	0,016	84,502	0,003	163,524	0,003	7471,259	5,070	1356,793
p-value	0,926		0,846		0,538		0,404	

Tabella 3. Analisi statistica relativa ai BPS su LYA a 20°C in naselli dell'Adriatico. Tesi 1 (ghiacciatura scarsa) versus tesi 2 (ghiacciatura ottimale). * Significatività non valutabile in quanto il valore della Tesi 2 al T6 è risultato costante.

Giorni di conservazione	T1		T3		T6		T10	
Rapporto tra le medie (=diff)	1,000		1,882		4,528		13,727	
IC95% (min;max)	0,040	24,940	0,108	32,713	-*	-*	0,188	1004,923
p-value	1,000		0,572		-*		0,166	

Tabella 4. Analisi statistica relativa alla CBT su PCA a 20°C in naselli del Tirreno. Tesi 1 (ghiacciatura scarsa) versus tesi 2 (ghiacciatura ottimale).

Giorni di conservazione	T1		T3		T6		T10	
Rapporto tra le medie (=diff)	1,960		0,981		1,831		2,727	
IC95% (min;max)	0,276	13,925	0,040	24,024	0,067	49,943	0,004	1942,128
p-value	0,433		0,989		0,638		0,579	

Tabella 5. Analisi statistica relativa alla CBT su LYA a 20°C in naselli del Tirreno. Tesi 1 (ghiacciatura scarsa) versus tesi 2 (ghiacciatura ottimale).

Giorni di conservazione	T1		T3		T6		T10	
Rapporto tra le medie (=diff)	3,030		1,723		1,974		2,959	
IC95% (min;max)	0,242	37,880	0,173	17,115	0,085	45,809	0,001	8223,249
p-value	0,324		0,583		0,581		0,616	

Tabella 6. Analisi statistica relativa ai BPS su LYA a 20°C in naselli del Tirreno. Tesi 1 (ghiacciatura scarsa) versus tesi 2 (ghiacciatura ottimale). * Significatività non valutabile in quanto il valore è risultato costante in entrambe le tesi.

Giorni di conservazione	T1		T3		T6		T10	
Rapporto tra le medie (=diff)	1,000		1,520		1,582		0,182	
IC95% (min;max)	-*	-*	0,131	17,592	0,002	1547,554	1,495	221058,47
p-value	-*		0,690		0,862		0,653	

I risultati della elaborazione statistica dei dati per il confronto fra il prodotto dell'Adriatico e quello del Tirreno mostrano differenze significative relativamente alla CBT per la tesi 2, come riportato nelle tabelle 7 e 8. In particolare risulta significativamente diverso (maggiore) il valore medio della CBT dei naselli dell'Adriatico al T1 rispetto a quello dei naselli del Tirreno al T2, e tale differenza risulta ancora discretamente rilevabile al T3. Si omettono per brevità i confronti che non hanno mostrato differenze significative.

Tabella 7. Analisi statistica relativa alla CBT su PCA a 20°C in naselli dell'Adriatico rispetto ai naselli del Tirreno, tesi 2 (ghiacciatura ottimale).

Giorni di conservazione	T1vsT2		T3		T6		T10	
Rapporto tra le medie (=diff)	30,577		6,163		0,312		6,792	
IC95% (min;max)	2,652	352,565	0,642	59,130	0,021	4,677	0,004	12085,635
p-value	0,016		0,094		0,298		0,475	

Tabella 8. Analisi statistica relativa alla CBT su LYA a 20°C in naselli dell'Adriatico rispetto ai naselli del Tirreno, tesi 2 (ghiacciatura ottimale).

Giorni di conservazione	T1vsT2		T3		T6		T10	
Rapporto tra le medie (=diff)	25,995		5,218		0,487		7,514	
IC95% (min;max)	2,624	257,538	0,404	67,400	0,021	11,392	0,008	6796,388
p-value	0,015		0,158		0,561		0,415	

CONSIDERAZIONI E CONCLUSIONI

Lo studio preliminare qui presentato consente di trarre alcune interessanti indicazioni sulla qualità microbiologica delle carni di nasello, in relazione alle modalità di manipolazione effettuate a bordo del peschereccio. Per quanto riguarda l'indice di contaminazione generica (CBT) sembrano non emergere differenze significative tra le tesi a confronto, ovvero i valori medi di CBT rilevati nel prodotto sottoposto a ghiacciatura scarsa sono risultati dello stesso ordine di grandezza o di poco superiori a quelli rilevati nel prodotto con ghiacciatura ottimale, sia nei naselli dell'Adriatico che nei naselli del Tirreno. Per contro sono risultate significative le differenze fra i valori medi della CBT nel prodotto dell'Adriatico rispetto a quello del Tirreno nella tesi 2 (ghiacciatura ottimale), in quanto il primo ha mostrato una contaminazione dell'ordine di grandezza di 3 log già al T1, mentre il secondo ha raggiunto tale entità di contaminazione solo al T6. Le differenze sono risultate pressoché nulle al T10, con valori di CBT pari a 4-5 log per entrambe le tesi ed entrambi i luoghi di produzione. I valori medi relativi ai BPS non sono risultati significativamente diversi nelle tesi a confronto e neppure in relazione alla provenienza.

Pur nella consapevolezza che il numero delle osservazioni effettuate è abbastanza limitato, sembra di poter dire che almeno fino a 3 giorni di appropriata refrigerazione post landing, la qualità microbiologica dei filetti di nasello non eviscerato (come d'uso in Tirreno) è sensibilmente migliore di quella del prodotto eviscerato (come d'uso in Adriatico), anche quando questo viene confezionato a bordo con ghiacciatura ottimale. L'utilizzo di due terreni per la valutazione della CBT, mostra che i valori rilevati su PCA e LYA sono sostanzialmente sovrapponibili. Il LYA, indicato anche come Iron Agar, consente di valutare simultaneamente la CBT e la quota specifica di BPS, in quanto contiene Tiosolfato di sodio e L-cisteina (11). Tenuto conto che la stima dei BPS risulta utile non solo per avere indicazioni rispetto allo stato attuale di spoilage, ma anche per valutare la rimanente shelflife del prodotto (12,13), il suo utilizzo risulta raccomandabile rispetto al comune PCA.

BIBLIOGRAFIA

1. Bremner H.A. 2002. Safety and Quality issues in Fish processing. Woodhead Publishing. http://knovel.com/web/portal/browse/display?_EXT_KNOVEL_DISPLAY_bookid=655&VerticalID=0
2. Mukundan M.K., Antony P.D.. Nair M.R. (1986). A review on autolysis in fish. Fisheries Research 4 (3–4), 259-269.
3. Shewan J. M. (1977). The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. In: Proceedings of the conference on handling, processing and marketing of tropical fish. Tropical products institute, London, 51–66.
4. Johnston W. A., Nicholson F. J., Roger A., Stroud G.D. (1994). Freezing and Refrigerated Storage in Fisheries. Stroud Series, FAO Fisheries Technical Paper 340, 143.
5. Hus H.H. (1995). Quality and quality changes in fresh fish. FAO Fishery Technical Paper 348. http://www.fao.org/docrep/v7180e/v7180e00.htm

6. Shawyer M., Medina Pizzali A. F. (2003). The use of ice on small vessels. FAO Fishery Technical Paper 436, 43.
7. Serratore P., Trentini M. (2005). Sardina pilchardus destinata al consumo di marinate crude: evoluzione della componente batterica durante la refrigerazione. XV Convegno Nazionale AIVI 16–18 giugno, Tirrenia, Italy, 411.
8. Lagrange F., Jark U., Etzel V., Feldhusen F. (2003). Einfluss des Ausnehmens auf die Qualität von frischem Seefisch. Archiv fur Lebensmittelhygiene 54, 65–70.
9. Oehlenschläger J., Rehbein H. (2001). Shelf life of gutted and ungutted ocean perch (Sebastes marinus and S. mentella) during ice-storage. In: Gudjonsson, A. and Niclasen, O. (eds.) Annales Societatis Scientiarum Faeroensis, Supplementum XXVIII, Torshaven, 51–61.
10. Skjerdal O.T, Lorentzen G., Tryland I., Berg J.D. (2004). New method for rapid and sensitive quantification of sulphide-producing bacteria in fish from arctic and temperate waters. International Journal of Food Microbiology 93, 325–333.
11. Gram L., Trolle G., Huss H.H. (1987). Detection of specific spoilage bacteria from fish stored at low (0°C) and high (20°C) temperatures. International Journal of Food Microbiology 4, 65–72.
12. Dalgaard P., Buch P., Silberg S. (2002). Seafood spoilage predictor-development and distribution of product specific application software. International Journal of Food Microbiology 73, 343–349.
13. Jørgensen B.R., Gibson D.M., Huss H.H. (1988). Microbiological quality and shelf life prediction of chilled fish. International Journal of Food Microbiology 6, 295–307.